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NATIONAL CENTER FOR RADIOLOGICAL HEALTH RESEARCH PROJECTS SUPPORTED BY GRANT FUNDS, JUNE 30,1968

Julius J. Sabo, et al

National Center for Radiolgical Health
Rockville, Maryland

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This publication provides a brief but comprehensive summary of research objectives, progress to date on the research, and the significance of the research to Public Health of the research project grant program of the National Center for Radiological Health. A list of publications resulting from this research funding, supported during the last fiscal year, are also provided.

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NATIONAL CENTER FOR RADIOLOGICAL HEALTH RESEARCH PROJECTS SUPPORTED BY GRANT FUNDS

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JUNE 30, 1968

Edited by

Julius J. Sabo

Sidney Leopold

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE • Public Health Service

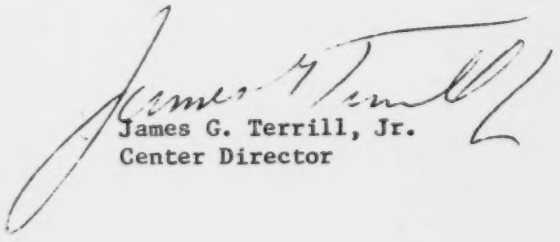
Environmental Control Administration • National Center for Radiological Health • Rockville, Maryland 20852

INTRODUCTION

The research project grant program of the National Center for Radiological Health is oriented to utilize the scientific competence of the academic community in solving defined and immediate problems existing in the field of radiological health as well as exploring and defining potential problem areas.

An effort is being made to have as comprehensive research projects coverage as possible in a problem area; the first example of this is in the program area of Methodology and Exposure Reduction for Dental and Medical Practices, specifically the discipline of Nuclear Medicine. Research project summaries on page 1 through page 16, with the exception of RH 00443 and RH 00562, are essentially directed towards this goal, and in addition RH 00042 on page 140, as well as several others would have contributions of vital information to this discipline. Many other examples of this type of coordination can be found in these summaries. The active research grants are presented by program areas, but the program areas are not mutually exclusive; as shown in the example previously given.

This publication, with its new format, was devised in order to allow the rapid dissemination of information on the research projects composing the grants program. The Principal Investigators were requested to provide the write-ups in a language that could be readily understood by persons not necessarily skilled in the same scientific discipline. The format that the Principal Investigators were asked to follow should provide a brief but comprehensive summary of the research objectives, the progress to date on the research, and the significance of this research with respect to Public Health. A list of the publications resulting from this research funding, supported during the last fiscal year, are also provided in order to allow this publication to act as a more valuable document for those with specific scientific interest. Acceptance of this publication will govern future publications of this nature, possibly with revisions in the format.



James G. Terrill, Jr.
Center Director

PREFACE

In order to make this a more useful publication, several modifications to the format used in previous years were made. In 1965, a publication of summaries of the Principal Investigators was distributed, and in 1966, a listing of the grant, with all publications that had resulted, was issued. In 1967, a listing of the grants by program area, which included a short descriptor of each grant and a list of all publications that were received in this office during the current fiscal year, was included.

In this publication, we have incorporated both of these formats; the summaries as prepared by the Principal Investigators with only essential editing by the staff, as well as a listing of all publications - where RH funding is reflected. The research grants are listed by program area and carry the following notes where required:

Note (1) - This grant did not receive Fiscal Year 1968 funds but was active.

Note (2) - This grant had terminated before Fiscal Year 1968, but publications giving credit for the earlier funding had recently been received.

Note (3) - Fiscal Year 1968 funds had been awarded for less than one year, therefore, summaries of progress could not be obtained in time for publication.

The two numerals listed in the grant number following the dash indicate the number of years the grant has received funding.

Since there are occasions when a Principal Investigator may be receiving grants support from several programs, publications are only listed if RH credit is given. Also, only those publications received after June 15, 1967 and before July 1, 1968 are listed, since earlier publications would have been listed in the previous document. Publications after July 1, 1968 will be listed in the next fiscal year document.

We wish to thank the Principal Investigators for the write-ups of the Research Objectives, Progress Report and Significance of Research. Where this material could not be obtained in time for publication, the staff included a brief description of the research project. This description was either abstracted from the Principal Investigator's Notice of Research Project (NRP), or in some cases, the NRP was included "in total" if not excessively lengthy.

The decision to follow the expanded format used in this publication was performed with the approval and assistance of Dr. Raymond T. Moore, Deputy Center Director.

We also wish to acknowledge the assistance of Mrs. K. Auth and Mrs. J. Jackson in the operation of the information retrieval system that was utilized in preparing this booklet, as well as their help in preparing the manuscript.

The editors:

Julius J. Sabo
Chief, Research Grants, OPPE
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National Center for Radiological Health

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DISCRIMINATION PROHIBITED--Title VI of the Civil Rights Act of 1964 states: "No person in the United States shall, on the ground of race, color, or national origin, be excluded from participation in, be denied the benefits of, or be subject to discrimination under any program or activity receiving Federal financial assistance." Therefore, the programs discussed in this publication like every program or activity receiving financial assistance from the Department of Health, Education, and Welfare, must be operated in compliance with this law.

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00350-01	IRRADIATION FROM ^{197}Hg AND OTHER RADIOISOTOPE TRACERS	YEN WANG, M.D., D.SC.(MED.) DEPT. OF RADIOLOGY UNIVERSITY OF PITTSBURGH PITTSBURGH, PENNSYLVANIA

Research Objectives: The kidney and whole body dose received from ^{197}Hg and ^{203}Hg labelled neohydrin will be adequately established by the initial stage of this project. This information will also become available for ^{99m}Tc, ^{51}Cr, ^{75}Se-methione and possible other isotopes. We further hope to determine the effect of plasma proteins binding to the biological half life and possible developement of new agents or compounds for accelerating the clearance of isotopes from the body and shortening the biological half life. Lastly, we hope to arrive at a correlation between the target organs counting and the whole body counting which will enable us to quantitate target organ concentrations.

Progress Report: Tests were performed using 450 rats and 12 dogs. Rat experiments were carried out to determine the specific irradiation contribution of radioactive mercurial compounds to the kidneys, liver, spleen, lungs, heart, brain, muscle, fat, bone, gonads, blood and plasma. These were determined using 0.1, 0.5, 1.0 mg/kg of mercuhydrin intraveneously as the renal blocking agents. These were given at 24 hours, 6 hours, 4 hours and 2 hours prior to the radioactive administration, at the time of radioactive administration, and 2 hours and 4 hours after the radioactive administration. These rat experiments have clearly establihsed that the optimal renal blocking condition is to give 0.5 mg per 1 kg of body weight of mercuhydin 4 to 24 hours prior to the administration of the radioactive mercurial compound. Under this regimen, 24 hours after the administration of the radioactive compound, only 11.05% of the radioactivity remains in the kidney. This compares to the 62.3% for the control animal which is 56.3% improvement. This gives the least radioactivity in all the organs and blood within the body. In contrast, with the inadequate blockage dose of mercuhydrin (less than 0.5 mg per kg body weight) and over blockage dose (more than

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00350 (Continued)

0.5 mg per kg of body weight) results in greater retention of radioactivity in all the organs and blood within the body, since there is apparently less excretion of radioactivity through the kidney. Therefore, it should be emphasized that an optimal condition of renal blockage with mercuhydrin is essential in reducing irradiation dose to the target organ (kidney) as well as to the rest of the body. This fact has substantiated by total body counting procedures.

The above observations have been reproduced with a larger animal (dog experiment).

Significance of Research: From the above, it is established that 0.5 mg/kg of non-radioactive mercuhydrin is the optimal dose for the rats weighing 150 grams and dogs weighing 15 kg in reducing the irradiation from radioactive mercurial preparations given intraveneously.

A 0.5 mg/kg dose of non-radioactive mercuhydrin should be given at least 4 hours prior to the administration of radioactive mercurial preparations in order to induce a maximal blocking effect on the kidneys. Doses larger than 0.5 mg/kg dose will be toxic to the kidney and impaire renal function.

Publications: None

RH 00416-03 — RADIATION DOSE FROM DIAGNOSTIC ISOTOPE PROCEDURES — EDWARD M. SMITH, SC.D.
SOCIETY OF NUCLEAR MEDICINE
NEW YORK, NEW YORK

Research Objectives: The primary objective of the Medical Internal Radiation Dose Committee of The Society of Nuclear Medicine is to provide the best possible estimate of the absorbed dose to patients resulting from the diagnostic or therapeutic use of internally administered radiopharmaceuticals. In order to achieve this objective, the Committee has, and is continuing to collect the necessary data on the distribution

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00416 (Continued)

of radiopharmaceuticals; the physical and nuclear decay scheme data on the relevant radionuclides; and physical, chemical, and biochemical properties, which may effect the biological behavior of the radiopharmaceuticals. The work of the MIRD Committee will continue to be necessary, as long as new diagnostic and therapeutic radiopharmaceuticals are developed.

Progress Report: The MIRD Committee was formed in October 1964, and its work has been supported, since the fall of 1965, by the National Center for Radiological Health. During that period of time, the Committee has held nine meetings, and its membership has grown from six to twenty members. The Committee members represent a multiplicity of scientific and medical disciplines from various universities; national laboratories; and private industry.

Recently, the Committee published its first report as a forty-page supplement to the Journal of Nuclear Medicine. This publication has been distributed to over 6,000 individuals in nuclear medicine, public health, and related disciplines in the United States and abroad. The contents of the Supplement included the first presentation of a unified method for calculating the absorbed dose, along with the physical data needed in this calculation. The approach, recommended by the Committee for calculating the absorbed dose, is a significant advance in this field; since it removes the limitations on the type and energy of the emitted radiations and anatomical model imposed by previous methods. This formalism is sufficiently flexible; so that one can compute the absorbed dose to a subcellular unit, for example, the nucleus of a cell, or to the total body of an individual, taking into account the individual's anatomical parameters.

The Committee's next Supplement is now in preparation, and will include a computer program to calculate the absorbed dose; additional physical parameters; and, of utmost importance, pamphlets presenting the detailed absorbed dose calculations for several radiopharmaceuticals, such as selenomethionine, a pancreatic scanning agent; neohydrin, a renal and brain scanning agent; macro-aggregated albumin, a lung scanning agent; and iodide, a thyroid evaluation agent. The Committee is actively working to collect

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00416 (Continued)

tissue distribution data for other radiopharmaceuticals, as well as additional physical data; so more realistic anatomical models may be used in the Committee's calculations.

Another important function of the MIRD Committee is to provide information with respect to the absorbed dose and tissue distribution of radiopharmaceuticals to federal, state, and local health and regulatory agencies, as well as cooperating with international agencies, whose mission is related to that of the MIRD Committee.

Significance of Research: The work of the MIRD Committee is vitally important; because one must be able to calculate the radiation absorbed by patients, in order to evaluate the potential radiation toxicity of radioactive materials. The quantity of energy deposited in tissue is referred to as the absorbed dose. It is a measure of the potential risk to the patient, and must be weighed against the probable gain from the diagnostic procedure. Calculation of the absorbed dose should be made for medical uses of radioactive pharmaceuticals, as well as for evaluating chronic environmental exposures and dangers from accidents involving radioactive materials. Until the work of the MIRD Committee, definitive information on both how much radiation is absorbed in a patient's tissue and how this information can be calculated accurately has not been available. The magnitude of the work of the Committee and the importance of this work will continue to grow; since the value of nuclear medical techniques, as a means of providing useful and definitive diagnostic information, is rapidly increasing.

Publications:

Official Publication of the Society of Nuclear Medicine, Inc. "MIRD - Journal of Nuclear Medicine", Supplement Number 1, Pamphlets 1-3, February 1968.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00428-02	PHYSICAL AND MEDICAL STUDY CS^{137} SUBSTITUTION FOR RADIUM	CHARLES G. AMATO, M.Sc. DEPT. OF PHYSICS MANHATTAN COLLEGE BRONX, NEW YORK

Research Objectives: The goal of this work is the generation of information which will encourage the replacement of Radium by radiotherapists with the radioisotope Cs-137. This step will well improve the safety of treatments requiring the use of Radium since Cs-137:

1) decays to a stable daughter;
2) is readily available as an inert cermanic;
3) does not give rise to gaseous products with their concomitant inhalation risk.

To meet this objective it is necessary to:

a) demonstrate the safety of Cs-137 microspheres;
b) demonstrate the adaptability of existing apparatus and techniques for use with Cs-137;
c) develop an isodose atlas similar to that currently used for Radium so treatments may be planned.

Progress Report: Twenty-seven Cs-137 needles were obtained comparable in loading and loading distribution to Radium needles currently used in certain radiotherapy procedures (brachytherapy). Gross radioactivity content for each Cs needle was verified by using properly calibrated ion chambers. Auto-radiographs were also taken of each Cs needle to provide additional verification. These radiographs were "read" by automatic scanning densitometers. Results indicated in some cases a need for an improved loading distribution; this situation was promptly corrected by the vendor.

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00428 (Continued)

Dosimetry evaluation of the dose distribution pattern in 24 combinations of needle type, needle number and implant planes were made using minature solid state dosimeters (radiophotoluminescent glass). Three hundred to 400 dosimeters were exposed for each implant producing a point by point dose distribution pattern. These distribution patterns were confirmed using four color film dosimetry.

The dose distributions were compared to those published for Radium needles (Patterson-Parker or Quimby tables); results indicate the distribution given by Cs-137 needles is within $\pm$ 10% of that predicted for Ra needles containing an equivalent amount of radioactivity. These results support the contention that if the Cs needle vendor calibrates the needles in terms of Ra equivalent the existing Tables may be used directly to plan treatments.

Significance of Research: The following conclusions have been drawn based on the experimental data:

1) Cs-137 needles can be fabricated to the specifications required for human use with respect to loading and distribution;
2) Cs-137 needles are a preferred source since the use of Cs eliminates the safety disadvantages attending the use of Ra;
3) the half life of Cs-137 while much shorter than Ra is of sufficient duration to permit a long useable service life (in all probability no replacement will be required);
4) the lower photon energy of Cs-137 with its lower half value layer may lead to a small dose reduction in regions beyond the volume being treated.

Publications: None

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00431-03	RADIATION PROTECTION AND MEASUREMENT	LAURISTON S. TAYLOR, D.SC. NATIONAL COUNCIL ON RADIATION PROTECTION & MEASUREMENTS WASHINGTON, D. C.

Research Objectives: The NCRP's principal objectives are (1) to collect, analyze and disseminate information and recommendations about radiation protection and measurement and (2) to stimulate the exchange of ideas and promote cooperation among organizations concerned with radiation problems. Thus the Council seeks to formulate recommendations on radiation protection and measurements which represent the leading scientific thinking on these questions and be of substantial benefit to those utilizing radiation producing equipment or materials.

Progress Report: The activities of the Council center about the work of the currently active scientific committees. During 1967 these included the following:

SC-1 - Basic Radiation Protection Criteria
SC-2 - Permissible Internal Dose
SC-3 - Medical X- and Gamma-Ray Protection up to 10 MeV (Equipment Design and Use)
SC-4 - Heavy Particles (Neutrons, Protons and Heavier)
SC-7 - Monitoring Methods and Instruments
SC-9 - Medical X- and Gamma-Ray Protection up to 10 MeV (Structural Shielding Design)
SC-11- Incineration of Radioactive Waste
SC-13- Safe Handling of Bodies Containing Radioactive Isotopes
SC-16- X-Ray Protection in Dental Offices
SC-17- Veterinary X-Ray Protection
SC-22- Radiation Shielding for Particle Accelerators
SC-23- Radioactive Elements in Man

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00431 (Continued)	SC-24- Radioactive Nucleic Acids and Precursors SC-25- Radiation Protection in the Use of Small Neutron Generators SC-26- High Energy X-Ray Dosimetry SC-27- Specification of Radium Substitutes	

Each of these scientific committees was actively engaged in the preparation of material which is expected to be suitable for publication as an NCRP report. Significant progress was made by most of these groups.

Scientific Committee 3 completed work on a report to supersede the portions of NCRP Report No. 24, Protection Against Radiation from Sealed Sources (1960) and NCRP Report No. 26, Medical X-Ray Protection up to Three Million Volts (1961) dealing with equipment design and use. During the year the Committee completed a redrafting of the material in response to the Council members' comments on the previous draft. The redrafted version was submitted to the Members of the Council in July 1967 and with minor changes was approved for publication. The report was sent to the printers at the close of the year and will appear early in 1968 as NCRP Report No. 33, MEDICAL X-RAY AND GAMMA-RAY PROTECTION FOR ENERGIES UP TO 10 MeV--Equipment Design and Use.

Scientific Committee 9 made very rapid progress during 1967 on its formulation of a related report dealing with structural shielding design for medical uses of x rays and gamma rays. As the year drew to a close, the work was nearing completion and it would appear that the report can be submitted to the Council for review early in 1968.

Related to the work of these two scientific committees is the decision reached late in 1967 to constitute a new scientific committee to treat the problems of brachytherapy. Originally it had been determined that material on brachytherapy would be included in the reports being prepared by Scientific Committees 3 and 9. However, as the work of the two scientific committees progressed, it became apparent that the effort necessary

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RH 00431 (Continued)

to complete the brachytherapy material would delay publication of both reports. Thus the Council determined to initiate the preparation of a separate report dealing with this topic and a new scientific committee (No. 29) was constituted to undertake this task.

During 1967 Scientific Committee 13 completed the initial drafting of a report on precautions in the management of patients who have received therapeutic amounts of radionuclides. This draft was also reviewed by the Council members during 1967. Most of the Council members approved of the draft, but since a substantial number of comments were also received, it was deemed appropriate to refer these comments to the Sciertific Committee for further consideration.

Scientific Committee 7 on Monitoring Methods and Instruments completed its drafting of a report to supersede NCRP Report No. 10, Radiological Monitoring Methods and Instruments. This report was being prepared for submission to the Council for review at the close of 1967.

Scientific Committee 23 on Radioactive Elements in Man carried on a very active program during 1967. The Committee devoted its attention to the problem of strontium-90 in the body. It examined the current status of our knowledge of the retention of the radioisotope, the microdosimetry especially in bone, and the long term biological results of the irradiation of sensitive tissues.

Four other reports moved forward rapidly during 1967. These are the reports being prepared by Scientific Committee 4 on Heavy Particles (Neutrons, Protons, and Heavier), Scientific Committee 11 on Incineration of Radioactive Waste, Scientific Committee 16 on X-Ray Protection in Dental Offices, and Scientific Committee 17 on Veterinary X-Ray Protection.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00431 (Continued)

The current activities of three of the Scientific Committees listed above represent NCRP undertakings recently initiated. These include the work of Scientific Committee 25 on Radiation Protection in the use of Small Neutron Generators, Scientific Committee 26 on High Energy X-Ray Dosimetry and Scientific Committee 27 on Specification of Radium Substitutes. Scientific Committee 25 began its work in March 1967 and directed its attention to the radiation protection problems involved in the use of small neutron generators in such applications as chemical analysis, nuclear physics, radiobiology, neutron radiography, oil well logging and teaching. The intent is to develop a guide to good practice in the use of low voltage neutron generators. Scientific Committee 26 is concerned with the problems of measurement and dose specification related to the clinical use of beams of high energy X-rays from 2MV to approximately 100 MV. The committee begain its work at a meeting held in June 1967. Scientific Committee 27 began its work in May 1967, devoting its attention to the problems involved in the specification of radionuclide sources used as substitutes for radium and radon. The committee began be giving immediate attention to Cesium-137 but is expected to take up other radionuclides later.

Significance of Research

The work of the NCRP has a significant impact on almost all activities in the United States which utilize or create radiation. The recommendations of the Council are important to radiation users-medical, industrial and government; to the general public; and to other state, national and international groups concerned with radiation matters.

The recommendations promulgated by the Council provide the scientific basis for radiation protection efforts throughout the United States. Individuals in industrial organizations employing radiation sources turn to these recommendations to be sure that their equipment and practices embody the latest concepts of protection. Non-government groups concerned with improving protection efforts and disseminating information on radiation protection look to the Council for guidance. Governmental

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00431 (Continued)

organizations utilize the NCRP's recommendations as the scientific basis of their protection activities. The NCRP also works closely with various international bodies concerned with radiation protection.

Similarly the work of the NCRP on measurement of radiation has found broad application throughout the United States and the world. Effective dissemination of information about radiation properties and effects requires that the measurement techniques employed and the quantities and units used be comparable throughout the United States and the world. The Council contributes to this goal by formulating and publishing the consensus of scientific opinion on various measurement problems.

Publications:
National Council on Radiation Protection and Measurements Report No. 33, "Medical X-Ray and Gamma-Ray Protection for Energies up to 10 MeV, Equipment Design and Use." February 1968.

RH 00443-15 PRECISION DOSIMETRY IN CLINICAL RADIOLOGY

JOHN HALE, PH.D.
DEPT. OF RADIOLOGY
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PENNSYLVANIA

Research Objectives: A long range aim of our research is to explore exhaustively the contribution of the discipline of radiological physics to the field of patient exposure and image quality control in diagnostic radiology. Specifically there are three areas of interest: (1) Analysis of Complete System Functions; (2) Analysis of System Variables; (3) Quality Control of Systems Performance.

Progress Report: Our initial experience with problems in system evaluation and quality control of radiographic equipment was obtained in the course of a careful study of cinefluorographic apparatus. These investigations resulted in a series of reports. The first, "Patient Dose, Film Illumination, and Radiation Transfer Function"

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00443 (Continued)

by J. Hale, D. L. George, R. D. Epperson, and T. A. Tristan was published in the book "The Reduction of Patient Dose," R. D Moseley, and J. H. Rust,(Charles C. Thomas, 1963, Pub.) This paper attempted to cope with the problems of establishing a meaningful calibration of apparatus equipped with automatic exposure control of both T.V. viewing and cinefluorography. The second paper in this series "Relative Patient Dose with 35 mm and 16 mm Camera Systems" by J. Hale, R. D. Epperson, D. L. George, and T. A. Tristan (Am. J. Roent., Radium Therapy, and Nuclear Medicine 92, 1192, 1954) is an example of the thorough analysis of one variable in a complete system.

It is surprising that very little effort has been directed to physical measurements in the diagnostic X-ray region. It has turned out, accordingly, that a major direction in our research thus far has been instrument development. "Exposure Meter and Phantom Materials" by J. Hale and D. L. George (Am. J. Roent., Radium Therapy and Nuclear Medicine 92, 1188, 1964) discusses the design and construction of a high precision wide range exposure meter that was required to measure accurately the very low exposure levels at the entrance plane to an image intensifier operating under clinical conditions. The second part of the paper discusses the problems of simulating patients of various thicknesses with phantom materials. The development of an improved technique for calibrating the peak operating potential of X-ray tubes by external measurement of the X-ray beam was published by P. Bloch and J. Hale as "Calibration of Potential of X-ray Generators in the Diagnostic Region" (Physics in Medicine and Biology 11, 577, 1966). We are presently deeply involved in the further development of a solid state radiation detector that can measure both radiation exposure and photometric luminence. An early model of this device has already been extremely valuable in both routine radiation safety survey work and in the determination of the conversion efficiency of radiation-to-light transducers. A preliminary report on this instrument was given by P. Bloch in the Bulletin of The American Physical Society 12, 643, 1967.

Finally, there are large areas in the physics of diagnostic radiology where basic

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00443 (Continued)

physical information is lacking. An example of this is data with regard to the first and second half value layers of X-ray beams in the diagnostic energy regions. These measurements had never been made with a diagnostic type radiographic tube and the difference in self-filtration introduced by the greater target angle of a diagnostic tube, as compared with a therapy tube, is a significant parameter in the experiment. These data were obtained in our laboratory and published in a paper "Homogeneity Factor for Pulsating Potential X-Ray Beams in the Diagnostic X-Ray Region" by J. Hale (Radiology 86, 147, 1966).

Significance of Research: In the past, most radiological physics attention to diagnostic radiology has been in the area of radiation safety. This research project is also intimately involved in the reduction of necessary exposure to the patient. On the other hand, and equally important, is the production of clinical information of value to the physician.

The long range objectives of our research are to establish procedures and methodology to perform complete analysis of diagnostic radiological equipment performance. This objective will entail the development of both new instrumentation and new methods of analysis. Parallel with this effort will be the development of methods for technically evaluating the effect of any system variable on patient dose or image quality. Finally we plan to identify the significant parameters to establish bench marks in developing protocols for the routine quality control of overall radiological diagnostic system performance. These procedures are necessary to ensure that the maximum value of the ratio of clinical information to patient dose is achieved and maintained.

Publications:

Bloch, P., "Instrument for Routinely Measuring the Efficiency of a Fluoroscopic Screen." Abstract AJ2, Bulletin of the American Physical Society 12, 643, 1967.

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(3)RH 00521-01	CANCER THERAPY BY INTERSTITIAL AND INTRA-CAVITARY RADIATION	BASIL S. HILARIS MEMORIAL HOSPITAL NEW YORK

Research Objectives: Interstitial and intracavitary radiation are of great importance in cancer management. There is no new modality in prospect which promises to replace either of them in the treatment of cancer. Despite the generally accepted usefulness of interstitial and intracavitary radiation, there is considerable reluctance of most clinicians to use these techniques because of the radiation exposure problem.

In order to significantly decrease or even eliminate radiation exposure and at the same time achieve more effective interstitial and intracavitary radiation therapy, the development of new isotope sources and techniques is proposed in this investigation. In the field of interstitial radiation therapy the use of low energy seeds is proposed as the most promising substitution for the presently used high intensity radioisotopes. In the field of intracavitary radiation therapy the use of "remote afterloading" with small high intersity sources moving during the treatment is outlined. This technique promises to eliminate all radiation exposure to personnel and make intracavitary radiation as safe and simple as X-ray therapy.

The Department of Medical Physics will place its major effort during this investigation on the problems of dosimetry and radiation exposure.

The clinical evaluation of the beneficial effects as well as the side reactions of interstitial and intracavitary techniques and the radioisotopes involved will be carried out.

Publications: None

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(3) RH 00523-01	ABSORBED DOSE DUE TO INTERNAL RADIATION EMITTERS	GORDON L. BROWNELL DEPT. OF MEDICINE MASSACHUSETTS GENERAL HOSPITAL BOSTON, MASSACHUSETTS

Research Objectives: The aim of this project is to investigate advanced methods of dose calculation and to compare the results of these calculations with experimental observations. Gamma-ray dose calculations are based principally on Monte Carlo methods of gamma-ray diffusion. Several existing programs will be extended to permit calculation of gamma-ray dose for a wide variety of geometrical conditions and for inhomogeneous media and for interfaces.

The development of a special Monte Carlo program providing spectral information will permit a wider range of application than has been possible in the past.

The experimental verification of dose calculation methods has been largely lacking in the past. Experiments will be performed to confirm Monte Carlo calculations of dose in various configurations including nonhomogeneous media and media containing interfaces.

Publications: None

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(3) RH 00532-01	IN SITU ASSAY OF RADIOACTIVITY FOR DOSE CALCULATIONS	EDWARD M. SMITH DEPT. OF RADIOLOGY UNIVERSITY OF MIAMI MIAMI, FLORIDA

Research Objectives: The objective of this research proposal is to quantitate the spacial and temporal distribution of radiopharmaceuticals in man so that more reliable absorbed dose calculations can be made.

Publications: None

(3) RH 00562-01	THE EFFECT OF X-RAY SPECTRUMS ON RADIOGRAPHIC QUALITY	ROBERT J. SCHULZ DEPT. OF RADIOLOGY ALBERT EINSTEIN COLLEGE OF MEDICINE BRONX, NEW YORK

Research Objectives: Almost all radiographic X-ray equipment is operated with 2.5 mm of aluminum filtration. This filtration allows for a wide spectrum of X-rays to be incident upon the patient and the width of these spectrums increases with kilovoltage. It appears reasonable, and preliminary experiments bear this out, that there are optimum X-ray spectrums for each type of radiographic examination; optimum in the sense of minimal patient exposure and maximum radiographic quality.

Our research objectives are to measure the X-ray spectrums that result from the use of various high atomic number filters whose absorption characteristics will markedly reduce the range of X-ray energies present in diagnostic X-ray beams. These "monochromatic" beams will then be evaluated in terms of the radiographic quality of radiographs they produce and the level of patient exposure.

Publications: None

METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(3) RH 00563-01	TV IMAGE STORAGE, ELECTRONIC MEMORIES AND RADIOLOGY	WILLIAM B. MILLER, JR. EMORY UNIVERSITY SCHOOL OF MEDICINE ATLANTA, GEORGIA

Research Objectives: The proposed research involves several investigations pertinent to recent developments in the fields of television image storage, electronic memory systems, and diagnostic roentgenography. The investigations include fundamental physics research for the determination of systems characteristics, the adaptation of computer techniques to the production, storage, and retrieval of images, and the performance of a series of controlled clinical trials to test operational hypotheses.

It is the major aim of the investigators to obtain the necessary fundamental data on a new and unique x-ray television method of visualization which will lead to new concepts of diagnostic radiology with emphasis on significant dose reduction to the patient and environment.

Publications: None

DEVELOPMENT OF DOSIMETRY, INSTRUMENTATION AND SYSTEMS

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00366-05	ENVIRONMENTAL RADIOACTIVITY	RICHARD I. WELLER, PH.D. DEPT. OF PHYSICS FRANKLIN AND MARSHALL COLLEGE LANCASTER, PENNSYLVANIA

Research Objectives: The object of this project is the development of suitable instrumentation for the simultaneous observation of the basic components of natural environmental radioactivity. This study is to furnish complete information on man's background radiation dosage. Ideally, what is sought is an absolute determination of the alpha, beta and gamma radiation background in which man lives. The simultaneous variations in the natural and man-made radiation components are to be investigated and correlations sought with time and meteorological conditions.

Progress Report: Prior to the construction of sensitive radiation detectors, suitable materials were sought containing minimal inherent radioactivity. It was found necessary to institute an experimental study. The results of this investigation indicated which materials were most satisfactory, and these results were published. This preliminary study also led to the positive identification of the radiocontaminant in ordinary lead, and the latter information was also published. Environmental radiation detectors have been developed and are being installed for use. A surplus trailer van was modified to house these detectors and serve as a field instrumentation station. Meteorological instruments and suitable data-recording apparatus have been secured for assembly into an integrated unit.

Significance of Research: The results of this project should provide accurate measurements of the exposure of man to natural radioactivity and cosmic rays. Radiation health problems of the general population may be correlated with weather characteristics. By furnishing instantaneous data on the individual radiation components in the environment, low-level and long-term radiation dosages can be assessed. Downwind fallout patterns and the consequent uptake of radioactivity in foods may be

DEVELOPMENT OF DOSIMETRY, INSTRUMENTATION AND SYSTEMS

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00366 (Continued)

predicted. The measurements of the components of natural radioactivity should furnish improved basic knowledge upon which to evaluate the radiation effects of various proposed peaceful and military sources of radiation.

Publications: No additional publications.

(1) RH 00404-04

DIGITAL COMPUTING TECHNIQUES IN RADIOLOGICAL PROBLEMS

JOHN L. HOWARTH, PH.D.
DEPT. OF PHYSICS & ASTRONOMY
UNIVERSITY OF NEW MEXICO
ALBUQUERQUE, NEW MEXICO

Research Objectives: The application of computer techniques to radiological problems with particular reference to the following:

I. The computation of doses near boundaries in inhomogeneous media irradiated with ionizing radiations, with particular reference to soft-tissue cavities in bone.

II. The development of mathematical models for the growth and dissemination rates of malignant tumors.

III. Calculations of the gamma-ray energy absorbed in bodies of different shapes containing distributed radioactive materials, and of other miscellaneous data of interest in radiology.

Progress Report:

I. Computations have been made of doses near boundaries in inhomogeneous media irradiated with X-rays of various energies. Results for the case of soft tissue cavities in bone have been published "Calculation of the Absorbed Dose in Soft-Tissue Cavities in Bone Irradiated by X-rays", Radiation Research, 24:158-183, 1965; "Calculations of the alpha-ray absorbed dose to soft tissue cavities in bone",

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

(1) RH 00404 (Continued)

Brit. J. Radiol. 38:51-56, 1965). These calculations have been extended to cover continuous X-ray energy spectra, and work is in progress on assessing the error involved in assuming that an X-ray beam can be considered equivalent to a suitably chosen monoenergetic beam, and on possibilities for comparing these continuous spectrum calculations with experimental data.

II. The development of mathematical models for growth and dissemination rates of malignant tumors. Some preliminary studies based on data obtained from collaboration with the late George W. Blomfield, F.R.C.S., Medical Director of the Sheffield National Center for Radiotherapy, were reported in a paper presented at the 1st International Conference on Medical Physics in Harrogate, England, in September, 1965. Further work on gathering of data is in progress.

III. Calculation of miscellaneous data of interest in radiology. Calculations have included curve fitting of the data of Nelms (Ann T. Nelms, National Bureau of Standards Circular 577 (1956) and Supplement (1958)) on ranges of electrons and positrons in various materials. This data has been made available through the International Atomic Energy Agency. Similar curve-fitting has been carried out on more recent data on electron ranges M. J. Berger and S. M. Silzer, "Tables of energy losses and ranges of electrons and positrons", NASA SP-3012, 1964).

Some calculations on the gamma-ray energy absorbed by bodies of various shapes and sizes containing distributed radioactive isotopes have been made, including a solution for the case of a gamma-emitter uniformly distributed in a cylindrical annulus.

Significance of the Research:

I. The dosimetry in inhomogeneous media is of importance in radiation therapy and in health physics since many human organs are inhomogeneous with respect to interaction with radiation. The case of bone is particularly important and the results of these

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(1) RH 00404 (Continued)

studies are relevant to the choice of radiation quality in radiation therapy and to the assessment of health hazards both for external radiation and for radioisotopes deposited in bone.

II. An understanding of growth and dissemination patterns of malignant tumors may throw light on their basic mechanisms, and may provide the clinician with better information on which to base a prognosis. It is hoped that this would be of value in deciding on the best mode of treatment.

III. Studies in the third category are generally for specific problems. For instance, the calculation for the cylindrical annulus was made in connection with a study (with Dr. R. L. Watts, UNM School of Medicine) on ^{125}I-labelled dye retained in the lipid layer inside the arterial wall. This study is aimed at a clinical method of estimating the extent of athero-sclerosis in humans.

Publications: No additional publications.

RH 00470-02 — COULTER COUNTER ASSAY OF BONE MARROW RADIATION INJURY

HUN LEE, M.A.
DEPT. OF SURGERY
INSTITUTE OF MEDICAL SCIENCES
SAN FRANCISCO, CALIFORNIA

Research Objectives: Our proposed goal for this study is the development of a rapid screening test for the evaluation of radiation injury of bone marrow for individuals involved in a large nuclear accident. Our previous studies on the radiation effect on bone marrow revealed that definite changes occur in the bone marrow cell-size distribution following irradiation. Using a Coulter counter as a tool for the cell-size measurement, we have pursued our goal according to the following scheme since the beginning of the project in June, 1967.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00470 (Continued)

Progress Report: 1. A study of the radiation effect on the ratio of the nucleated cells over the non-nucleated cells in the marrow: The work is completed and published. In this study, we found that the ratio of the marrow cells over the red blood cells drops rapidly with the increase of radiation dose at the sublethal level. The drop slows down at the low and mid-lethal doses and levels off at high lethal doses. Thus the ratio is dose dependent up to a limit. This ratio may be used as an index for the classification of radiation injury.

2. A study of the normal cell-size relationship between the circulating blood, bone marrow and other organs where certain circulating blood cells may originate: The work is completed and submitted for publication. The purpose of this study is to establish a normal cell-size relationship between the circulating blood cells, and cells in the bone marrow and other lymphoid organs where the circulating lymphocytes might be produced. When their normal size relationship is established, any deviation from this relationship in the circulating blood after irradiation probably can be taken as a reflection of the injury received by the source organs, especially the bone marrow. In this study, an interesting relationship between the circulating blood cells and bone marrow cells was established. We found that the circulating red blood cells probably have two different size groups. The normal non-irradiated marrow red blood cells have a size distribution correspondent to that of the large circulating red blood cells, while the irradiated marrow red blood cells have a size distribution correspondent to that of the small red blood cells. The circulating lymphocytes were also found to be larger than those in the marrow and the lymphoid organs. The circulating neutrophiles, however, have the same size as those in the marrow. This interesting relationship may be of value to serve as key to any changes in their normal relationship.

3. A study of the radiation effect on the blood leukocyte size distribution as the manifestation of radiation injury of the marrow: The work is completed, and a manuscript for publication is in preparation. In this study, we found that the blood leukocytes (or white blood cells) size-distribution pattern changes with the radiation dose. The changes reflect or correspond with the injury received by the marrow during

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RH 00470 (Continued)

the first 24 post-irradiation hours. The responses of different individual animals, as revealed in the changes of their leukocyte distribution patterns, vary. It is possible that these variations are related to the chances of survival and course of recovery of these animals. For a study of the possible relationship between the two, we initiated the following study:

4. A study of the relationship between the chances of survival and course of recovery of the individuals irradiated, and the blood leukocyte (white cells) size-distribution patterns of these animals: In order to study this relationship, we cannot sacrifice the animals for blood samples as is necessary now when mice are used. We have to keep the same individuals alive after blood sampling to see how they fare after the radiation exposure. This is necessary if a reliable relationship between the two is to be established. Since mice have only a limited volume of blood, it is impossible to save the animal after the blood sample is taken. The leukocyte test requires 0.5 ml of blood which is a large part of the total blood volume of the mouse. For this reason, we are forced to shift to a larger animal, the rat, for the present phase of our study. A study of the normal rat marrow and blood cell-size distribution is now in progress.

Significance of Research: The significance of this research project can be summed up in a few words. After exposure to a lethal amount of radiation, different individuals receive injuries as a result of the radiation exposure in varying degrees. Some will die within a short time and some will survive. Up to now, there is no rapid method to determine whether hospitalization is necessary for each particular victim and what his chances are to survive and to recover. In a large nuclear accident, or war, when a large section of the population is involved, such a method is required in order to screen the victims. For this task the method must meet the requirement of speed as well as accuracy. The test under study by us is based on electronic instrumentation and automation which we hope may fulfill both.

Publications: None

DEVELOPMENT OF DOSIMETRY, INSTRUMENTATION AND SYSTEMS

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(3) RH 00536-01	THERMOLUMINESCENCE OF RUBY AND IONIC CRYSTALS	WILLIAM G. BUCKMAN, PH.D. DEPT. OF PHYSICS WESTERN KENTUCKY UNIVERSITY BOWLING GREEN, KENTUCKY

Research Objectives: Some materials thermoluminescent properties have made them useful as radiation dosimeters and they are complementary to and competitive with the film badge for personnel radiation dosimetry. The thermoluminescent yield and spectral emission of the ruby and ionic crystals will be measured. The spectral emission dependence on temperature, while the luminescence is produced by different modes of excitation, will be determined.

Publications: None

STUDIES OF NUCLEAR PLANT MONITORING, SURVEILLANCE, AND DESIGN CHARACTERISTICS

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(2) RH 00077-06	EFFECTS OF RADIUM ON STREAM BIOTA BELOW URANIUM MILLS	WILLIAM F. SIGLER, PH.D. DEPT. OF WILDLIFE RESOURCES UTAH STATE UNIVERSITY LOGAN, UTAH

Descriptor: Major emphasis was placed on field collections in the area of radium contamination. Various ecological factors and physical parameters were measured. Studies were conducted in areas above and below a newly opened uranium processing mill to study the early pollution effects of mill wastes. Laboratory studies were made to determine uptake of radium by algae. Collection of channel catfish was performed at various points on the Dolores River below Uravan, Colorado, in an effort to assess the degree and frequency of intense pollution.

Publications:
Sigler, W. F. "Uranium Mill Wastes as Stream Pollutants." Utah Science, Vol. 28, No. 2, June 1967.

RH 00412-03	ECOLOGY OF RADIONUCLIDES FROM A REPROCESSING PLANT	N. IRVING SAX, B. S. HEALTH RESEARCH, INC. ALBANY, NEW YORK

Research Objectives:

A. To very thoroughly elucidate the dispersion patterns of stack emitted radioactive pollutants from a specific source in order to develop a simple but sophisticated surveillance technique generally applicable to sources of environmental contamination.

B. To study the hydrological distribution of radioactive environmental pollutants from a specific nuclear reprocessing plant source discharging into the relatively simple stream system which drains the site for general application to environmental pollution control.

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(Continued)

C. From our studies of plant discharges, the ambient meteorology and the eco system surrounding it, to develop a system of natural indicators of environmental contamination as well as a network of optimized sampling points and where necessary, radio-analytical techniques compatible with the problem of environmental pollution control.

Progress Report: We have made significant progress in each of the above cited research objectives.

Although research objective A proved much more difficult than we had anticipated, we were fortunate to be able to develop a whole new technique around stack emitted tritiated moisture in time to do the job.

Since the area surrounding this plant is largely undeveloped with few roads and difficult topography, we were forced to develop a simple, effective, wide-area sampling technique. This work also resulted in additional technical reports which have been submitted to appropriate journals for publication.

Our work on ^{85}Kr indicates that this is a potentially important radioactive air pollutant. Also, because of its chemical inertness and long half-life, it may be useful as a tracing agent for air pollution control agencies as well as metorologists.

A careful search of the literature revealed that there was no simple, reliable, analytical technique for the analysis of ^{85}Kr in air at normal levels of concentration i.e., 1-10 pCi/m^3. Also existing methods for sampling air for ^{85}Kr were mainly of a laboratory nature, not well adapted for application to the field particularly in as rugged a terrain as we were facing. At the very least, a sizeable power source was required to run an air compressor for one of the sampling techniques and even this

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was out of the question for our study. To solve these problems, we initiated a research program which led to the successful development of a very sensitive analytical technique. Now that we had a really sensitive analytical technique, we were able to drastically simplify the field sampling procedure which would in any case be necessary before wide-spread surveillance for ^{85}Kr could be undertaken. This technique has been thoroughly tested and we find it produces analytical data with a precision of $\pm$10% and is entirely adaptable to field use since it requires only a size 10-20 plastic bag available commercially for about 40 cents each, complete with a nozzle. The bag is inflated either by means of a hand operated bellows or a pocket-sized battery-operated automobile vacuum cleaner which may also be operated from an automobile storage battery via the cigarette lighter. The inflated bags may be returned to the laboratory for analysis by means of standard cardboard shipping containers.

The mobile meteorological laboratory, which we designed and built for determining the concentration of condensation nuclei in field air samples, has worked out very well. This could be a very sensitive approach to stack emitted air pollution mapping.

With it we have made pre-operational condensation nuclei maps over much of the area about the plant under study. As a result of the success of our tritium work, we can locate the plume at ground level and can undertake the second phase of the condensation nuclei study which involves mapping areas affected by the plume as compared to those not affected to determine empirically the sensitivity of such mapping as a means for stack emissions surveillance. The advantage over tritium, krypton or iodine sampling is that the sample while non-specific for type of pollution involved, can be taken and analyzed within a few seconds and may be useful as a gross detection mechanism to indicate places and times for more specific sampling.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

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Research objective B is coming along very well. Our idea of placing this study at the site of a nuclear fuels reprocessing plant in order to make use of emitted radionuclides as tracers is working out. Although the plant is in the early stages of operation and even though the operation is intermittent and the amount of activity released to the environment relatively small, the great sensitivity of radiological tracing has been effective. We have established sampling sites along the Buttermilk and Cattaraugus Creeks at which points stream data, meteorological data and eco data are taken. We have been able to trace the flow of tritium as tritiated water along the water courses and detected it in samples of wildlife population which live near these waters. We have been able to demonstrate uptake of Co, ZrNb, Cs, RuRh and Sr isotopes by silts and bottom muds, fish, algae and some vegetation. This work has been summarized into a report and submitted for publication.

Now that the plant is operating with fewer interruptions and at higher levels of thruput, our data is valuable for comparative purposes and our familiarity with the area and established sampling points could prove very useful to future studies.

Research objective C since it depends upon the progress of A and B is in an early stage of completion. We have found a number of good candidates for indicating mechanisms of environmental pollution. These include silts, two varieties of fish and algae. The use of imported clams as reported under B above looks very promising since they are easily obtained free of contamination, can be held immersed in the streams under study and seem to be sensitive indicators of stream contamination. This approach may be original and we intend to follow it up vigorously.

Significance of Research: Since this work is contributing directly to the science of environmental pollution, its public health significance is obvious. Although we are dealing with a nuclear fuels reprocessing plant, the relationships we derive are of wide and general usefulness to the whole area of environmental pollution control.

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However, now and in the immediate future this work constitutes a significant contribution to the control of radiological health hazards to populations in the vicinity of units of our rapidly expanding nuclear technology. Simplified, sophisticated control will lead to better measure urgently needed to keep population exposures down to a minimum.

Publications:

Daly, J. C., Manchester, A. V., Gabay, J. J. and Sax, N. I. "Tritiated Moisture in the Atmosphere Surrounding a Nuclear Fuel Reprocessing Plant." Radiological Health Data and Reports 9, No. 7, 341-346 (1968).

Sax, N. I. and Gabay, J. J. "A Surveillance Technique for Tritiated Moisture in the Atmosphere." Proceedings of the Symposium on Environmental Surveillance in the Vicinity of Nuclear Facilities (Jan. 24-26, 1968, Augusta, Ga.), January 1969.

Gabay, J. J. and Sax, N. I. "A Simplified Technique for Stack Dispersion Mapping with Tritium." Environmental Research 2, No. 1 (1968).

Sax, N. I., Denny, J. D., and Reeves, R. R. "Determination of Low Level Krypton-85." Analytical Chemistry 40, No. 10 (1968).

Sax, N. I., Reeves, R. R., and Denny, J. D. "Surveillance for ^{85}Kr in the Atmosphere." Radiological Health Data and Reports 9, No. 11 (1968).

STUDIES OF ENVIRONMENTAL PATHWAYS

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00030-06	ENVIRONMENTAL EFFECTS ON THE CONTENT OF CS-137 IN MILK	ROBERT C. PENDLETON, PH.D. DEPT. OF MOLECULAR & GENETIC BIOLOGY UNIVERSITY OF UTAH SALT LAKE CITY, UTAH

Research Objectives: This study was designed to determine the environmental factors which are responsible for the observed seasonal and geographic differences in content of ^{137}Cs and ^{90}Sr in milk, animal fodders and humans and to determine the range of these variations in environments represented by selected dairy farms of Utah. Field studies were planned to test the validity and importance of factors affecting fallout accumulation which had been identified in the laboratory. The overall objective of the study was to determine how farming practices could be modified to lessen transfers of fallout to foods and man.

Progress Report: Assessment of the quantity of radioactive fallout materials in human diets, milk, animal fodders, soils, livestock, wildlife and people has been made during a six-year period at 55 dairy farms distributed over the major agricultural areas of Utah. Each farm has been studied intensively to determine the ecological characteristics that might affect the content of radioactive contaminants in the milk produced.

Environmental factors determined at each farm have included meteorology, topography, geography, soils, vegetation, and farming practices. More than 7,000 samples have been taken and analysed for radioactivity as well as for chemical and physical properties.

The following contributions to the understanding and control of fallout accumulation have been made from the information obtained:

1. The first state-wide evaluation of the distribution, contamination levels, human accumulation and probably radiation doses from Iodine-131 released by local tests.

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2. New computer methods for calculating levels of radioisotopes in soils, vegetation, wildlife, milk and humans; new methods for comparing the relative contribution of a single environmental factor as compared with interactions of multiple factors.

3. Particular combinations of environmental conditions and farming practices always lead to higher accumulation of fallout in milk; it is possible to predict the relative amount of radioactive materials in the milk produced on any farm by evaluating the presence and relative contribution of factors known to cause increased contamination levels.

4. Milk produced on farms, grouped in climatic regions, consistently shows contamination levels characteristic of the rainfall of that region. The amount of ^{90}Sr or ^{137}Cs in milk from a single farm remains constant relative to the levels in milk from other farms within a climatic region. The differences in contamination levels between farms in one region are only two to three fold, but may exceed ten fold between regions.

5. The State of Utah can be divided into a least six zones of fallout on the basis of levels in milk, and these zones follow a north-south and east-west pattern. Rain shadows cast by high mountains strongly affect the boundaries of the fallout zones.

6. The levels of contamination in crops usually follow a predictable ranking (high to low) as follows: meadow-clippings, grass hay, alfalfa (first, second and third crops), meadow-grass silage, oats silage, corn silage and grain.

7. The concentration of ^{137}Cs or ^{90}Sr in milk from a given farm can be predicted for any month of the year from the feeding pattern; annual cycles of milk content follow changes in crops fed.

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8. Body-burdens of ^{137}Cs for a farm family reflect levels in milk produced on that farm. An average three-fold increase in $^{137}Cs/K$ ratios in humans over the Cs/K ratios in milk has been observed in farm people since fallout levels have peaked. The magnitude and importance of relative ^{137}Cs half-times in humans have been established.

9. Levels of ^{90}Sr and ^{137}Cs in surface soils differ according to great soil group, increasing from soils of arid regions to dark soils of sub-humid areas. There is little correlation between precipitation and fallout levels in arid and semi-arid soils, but dark soils of the sub-humid climates show as much as 94% correlation.

10. Surface transport of fallout contaminants relative to slope has been measured on all farms, and rates of movement appear to depend on many environmental differences. Deposition of isotopes from irrigation water in poorly drained areas is creating "hot spot" fields that become increasingly contaminated each year.

11. Chemical and physical conditions in soils have little observable effect on the accumulation of ^{90}Sr or ^{137}Cs in crops, or, ultimately, milk. Contributions from other environmental factors such as drainage, feeding patterns or grazing time are so great that effects of soil conditions are obscured.

12. Changes in farming practices alter the output of radioactive materials in the milk, and relatively inexpensive modifications of fodders grown, crop rotation, and drainage can markedly reduce the quantity of radionuclides in dairy products.

13. Radioactivity in excess of 1 uCi/kg has been found in samples of dust taken from farm implements. Operators are exposed to secondary radioactive aerosols generated by harvesting machines from deposited fallout on crops.

Significance of Research: We have defined recognizable ecological factors that enhance the transfer of radionuclides to milk. Certain farms consistently produce milk that is higher in radioactivity than other farms in the same region. Factors responsible for

STUDIES OF ENVIRONMENTAL PATHWAYS

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RH 00030 (Continued)

this increase can be altered so that these farms can produce milk of far less radioactive content. Inhabitants of farms usually have body-burdens of cesium that are correlated with the content in the milk. Our studies have led to the development of specific recommendations that can be used by farmers and Health Departments to lessen hazards from fallout in the event of nuclear war or accident. Results from this project have led to numerous studies by other investigators on the safety aspects of atomic testing. An appreciation of both potential and past hazards has been generated as evidenced by state-wide investigations of the incidence of thyroiditis, thyroid cancer and other disorders possibly attributable to radiation. A direct result of our work on human cesium metabolism has been the initiation of studies concerning muscle disorders which may prove to be of fundamental importance.

Publications

Lloyd, R. D., Christensen, J. A., Church, B. W., Fisher, P. S., Mays, C. W. and Pendleton, R. C. "Polyspec: A Computer Program to Resolve Complex Gamma-Ray Spectra from Samples of Different Densities." Health Physics, Vol. 13, No. 8, August 1967.

Mays, C. W., Zundel, W. S., Lloyd, R. D., Wagner, W. W. and Pendleton, R. C. "Potassium and ^{137}Cs Studies in Patients with Muscular Dystrophy." Abstract Ac-4, Radiation Research, Vol. 31, No. 3, July 1967.

Lloyd, R. D., Zundel, W. S., Mays, C. W., Wagner, W. W. and Pendleton, R. C. "Enhanced Cs 137 Elimination in Pregnant and in Dystrophic Humans." Radiation Research, Vol. 27, No. 2, February 1966.

RH 00076-09 RADIOBIOLOGICAL RESEARCH ON MARINE ORGANISMS

H. BURR STEINBACH, PH.D.
MARINE BIOLOGICAL LABORATORY
WOODS HOLE, MASSACHUSETTS

Research Objectives: The funds derived from this grant go to support of the hot lab area comprised of ten inter-connecting rooms on the fourth floor of Whitman Laboratory.

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These rooms, equipped with appropriate counting devices as well as radiation sources, are made available to qualified investigators. The funds also defray the expenses of the radiation safety officer and his assistant.

Any qualified investigator registered at the MBL may use the facilities. Last year 120 senior scientists were served. Allocation of space at the MBL is made on the recommendation of a special committee of the Trustees of the Corporation.

Progress Report: Applications for research space are reviewed by a standing committee. This year 280 applications have been received to date. Two hundred and sixty assignments have been made. Of these, 106 have indicated their need for the hot lab facilities.

Roughly, the projects fall into three categories:

a. The use of such isotopes as ^{42}K for studying ionic fluxes, especially in excitable systems. Much work is done on the giant axon of the squid. The MBL is one of the few places in the world which can provide a reliable supply of this unique material.

b. The use of tritiated and ^{14}C intermediates in studying basic process during development. Extensive use is made of invertebrate ova from such animals as Arbacia and Spisula.

c. The use of labelled intermediates to study metabolic patterns. For example, the effects of insulin on carbohydrate metabolism, making use of the unique anatomical features of the goosefish.

By rough count, each of the above categories accounts for nearly a third of the users of the facilities. In addition, there are studies such as ecological investigation of uptake and distribution of isotopes within and between organisms.

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Significance of Research: The total program of the MBL consists of the individual programs of the nearly 300 independent investigators who use the facilities for periods ranging from two to twelve months of the year. As noted, last year 120 of these investigators were registered as using the hot lab facilities. Since much collaboration exists between individuals, the number of research projects was probably greater than the number of individuals registered.

Publications: None

(1) RH 00175-03 RADIONUCLIDES IN FLORIDA MILK

EDWIN G. WILLIAMS, M.D.
FLORIDA STATE BOARD OF HEALTH
JACKSONVILLE, FLORIDA

Research Objectives: From Federal and State studies, it is evident that although Florida receives somewhat less strontium fallout, the levels of cesium are consistently higher than those for the nation as a whole. The purpose of this research is to explain this finding. Influences of geographic location (within the state), herd drinking water, pasture versus "mixed seed", types of pasture, condition of pasture and soil types, are being studied and related to levels of radioactive materials in milk.

Progress Report: All data obtained in the progress of this work corroborates the initial observation that Florida milk does indeed contain more radioactive cesium and less radioactive strontium than is true for the nation as a whole. An independent study conducted by the Public Health Service indicated that Pangola grass was in some way associated with high cesium content of milk. The present study showed that even higher levels occur in some areas where there is no Pangola grass fed. No strong correlation was found between feeding practices and the cesium level. Both surface and well water were so low in radioactive substances as to be of no importance to the solution of the problem. Cesium levels in West Florida were found to be relatively low, those in the northeastern part were intermediate and those in the central part of the state were relatively high. This suggests some intriguing questions but they have not yet been

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explored. Although soil types in Florida constitute considerable admixture of the various series, general geographic areas seem to be sufficiently characteristic to afford a study of radionuclide levels in relation to soil types. Data are now being analyzed to this end.

Significance of Research: The significance of resolving the questions posed in paragraph one is related more to a better understanding of ecological biology which undoubtedly will have far reaching implications in the field of health than to an immediate remedial activity program for the correction of a recognized deviation which may affect health. Unfortunately, at the present time, it would seem that considerably more study will be necessary to arrive at meaningful conclusions.

Publications: None

RH 00307-04 BIOLOGICAL ENRICHMENT CHAINS OF RADIONUCLIDES IN FINNISH LAKES

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UNIVERSITY OF HELSINKI
HELSINKI, FINLAND

Research Objectives: The purpose of the project is to elucidate the limnological factors effecting the accumulation of the biologically most important radionuclides in fresh water organisms, especially fish. Food chains of ^{137}Cs from the global fallout are studied in 12 lakes of limnologically different types (from nutrient deficient ones to those rich in nutrients). In addition, isotope labelling experiments are carried out with ^{137}Cs, ^{85}Sr, ^{131}I, ^{140}Ba and ^{45}Ca in five lakes of different limnological types in order to determine quantitatively the changes with time in the distribution of the isotope between different compartments of the lake biosystem.

Progress Report: The studies of ^{137}Cs from the global fallout in 12 lakes of different limnological types showed that the lower the potassium concentration in the water the higher the ^{137}Cs concentration in the vegetation, bottom animals and fish. Potassium

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was shown to be by far the most important limnological factor effecting the enrichment of ^{137}Cs in the fish. By determining, in laboratory and field conditions, the excretion rate of ^{137}Cs in various fish species, it could be shown that the excretion rate is the second factor in importance effecting the ^{137}Cs body burden of the fish. The third and minor factor is the quality of food eaten by the fish.

It was found that the excretion rate of ^{137}Cs in fish follows a two-exponential equation: a smaller part, usually 10 to 30 per cent, of ^{137}Cs is excreted rapidly, showing a "half-time" of a few hours or days, the rest much more slowly with a half-time of weeks, months or even years. In most fish species the excretion rate is age-dependent being 2 to 3 times faster in young fish (less than 1 year) than in older fish. The excretion rate is also temperature dependent: at 15° C (summer temperature) the excretion is 2 to 3 times faster than at 5° C, which is the mean annual temperature of most Finnish lakes. The biological half-time of ^{137}Cs in fish (the slow component of excretion) varies from 10 to 500 days and this knowledge is an unavoidable prerequisite for any quantitative treatment of the factors effecting the ^{137}Cs body burdens in fish.

Further, it was shown by aquarium experiments that the direct uptake of ^{137}Cs through gills, is in practice, in fresh waters insignificant compared with the uptake in food. With the knowledge of the radioactivity of the fish and its food and excretion rate it was possible to calculate the food uptake of fish in natural lakes from their body burdens.

Although the studies of global fallout are important, these studies usually do not give the total balance of a radionuclide in a lake biosystem since the amount entering and leaving the system remains unknown.

By pumping known amounts of tracer isotopes into closed lakes it was shown that the limnological type of the lake also determines the route and rate of removal of the radioisotope from the lake biosystem. In a eutrophic lake ^{137}Cs was rapidly removed

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from the water into the bottom sediment by practically irreversible adsorption onto clay particles suspended in the lake from the shore banks. In an oligotrophic lake the removal was much slower and mainly took place into the moss and turf at the shore line. As the low potassium content in the oligotrophic lakes further accentuates the ^{137}Cs-uptake in the fish, the body burdens become up to three magnitudes higher than in turbid lakes, rich in nutrients, and up to two magnitudes higher than in clear watered lakes, rich in nutrients.

Total balance experiments with ^{131}I in a nutrient-deficient lake and in a lake rich in nutrients showed that iodine (the element, after correction for the decay) is slowly removed from the water. In the oligotrophic lake the half-time of the removal was about 45 days, in the nutrient-rich lake the removal showed two rate components: the half-time of the fast component, effected by the rich vegetation, was 6 days, that of the slow component about 50 days. The difference in the enrichment factors was small (about 2), probably due to the fact that the iodine content of the lake water does not follow the "trophic classification": the oligotrophic lake had a higher iodine content (3 ug/l) than the eutrophic one (2.4 ug/l). After one month the water activity in the nutrient-deficient lake was 1/25th of the original, but the fish contained then about 800 times more ^{131}I per unit weight than the water (6 times more than was the initial water activity).

Tracer experiments with ^{85}Sr have also been carried out in an oligotrophic (nutrient-deficient) and a eutrophic lake. In the first-mentioned lake strontium (the element, when corrected for the decay) was slowly removed from the water by a half-time of about 50 days. The effective half-time for the removal of ^{85}Sr-activity was 28 days. Plankton and moss reached an equilibrium with water in about 10 days, but fish did not reach equilibrium during the whole time of the experiment (5 months). The limnological nature of the lake has a much smaller effect on the enrichment factors of radiostrontium than on those of radiocesium.

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Significance of Research: This research presents a scientific basis for theoretical calculations of fish activities at different times after pollution in lakes of widely different limnological types. The main factors effecting the fish activities are:

1. the rate of removal of the radionuclide from the lake water (suspended inorganics, rich plankton, rich vegetation)
2. amount of carrier nutrients
3. physiology of the fish (excretion rate)
4. feeding habits of the fish (minor factors)

Quantitative determination of the magnitude of each of these factors has made possible a systems analysis approach to the problem and a scientific basis for the control of radiation hazards in the fresh water environment.

Publications

Kolehmainen, S., Romantschuk, H., Takatalo, S. and Miettinen, J. K. "Total Balance Experiments with Radioisotopes of Caesium in Three Lakes of Limnologically Different Types." 4th Scandinavian Symposium on Environmental Radioactivity, Oslo, October 1968.

Kolehmainen, S., Romantschuk, H., Takatalo, S. and Miettinen, J. K. "Total Balance Experiments with ^{131}I and ^{85}Sr in Lakes of Different Limnological Types." 4th Scandinavian Symposium on Environmental Radioactivity Oslo, October, 1968.

Kolehmainen, S., Romantschuk, H., Takatalo, S. and Miettinen, J. K. "Pollution Experiments with Cs-137 in Lakes of Two Limnologically Different Types." Report submitted to the International Congress on the Environmental Radiation Protection connected with the Development of the Pacific Use of Nuclear Energy, Toulouse, France, March 14-16, 1967.

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	GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1)	RH 00395-01	PEDIATRIC SIGNIFICANCE OF PEACETIME FALLOUT	Lee E. Farr, M.D. American Academy of Pediatrics Evanston, Illinois

Descriptor: This conference of leading pediatricians, radiologists and radiobiologists was called to determine the pediatric significance of peacetime fallout which included fallout activity in food, water and milk. Measures taken by various government agencies to alleviate problems, to disseminate quick, early information and actions that would go into effect, were also evaluated.

Publications:
"Conference on the Pediatric Significance of Peacetime Radioactive Fallout." Supplement to Pediatrics, The Journal of the American Academy of Pediatrics, Vol. 41, No. 1, Part II, January 1968.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00124-06	FISSION PRODUCT - SOIL ORGANIC MATTER COMPLEXES	RALPH E. FRANKLIN, PH.D. DEPT. OF AGRONOMY OHIO AGRICULTURAL RESEARCH AND DEVELOPMENT CENTER WOOSTER, OHIO

Research Objectives: Our objective is to evaluate the ability of soil organic matter to inactivate fission products in contaminated soil, thereby reducing the accumulation of these radioisotopes by plants used for human or animal consumption. Included in this primary objective is the gathering of information on the chemistry of soil organic matter-fission product complexes such as the availability to plants, movement through soil, and the turnover of the fission products through microbial activity.

Progress Report: Studies have been conducted on the fate of strontium 90 applied to soils. Both the organic matter and strontium 90 are strongly adsorbed by the soil. We have used relatively mild extracting agents in removing organic matter from soil for further studies so as to effect minimum alteration of the organic compounds. Two extractants have been used, hot water and sodium pyrophosphate. About 1 1/2 percent of the strontium 90 in our samples was removed in the hot water extraction. Three-fourths of the strontium was complexed by the polysaccharide fraction of the water soluble organic matter. The preliminary characterization studies of this material showed that the strontium and yttrium were complexed by compounds rich in phosphorus. Several buffers were compared for their effectiveness in obtaining electrophoretic separation of strontium and yttrium complexes. A borate buffer at pH 9 gave best separation. Evidence was obtained that microbial activity during laboratory incubation may seriously affect the reactivity of soil organic matter complexes. The polysaccharide-free soil organic matter also readily complexed strontium 90 and yttrium 90. One component was characterized as an acid salt of an aromatic amine. Blocking of carboxyl groups through methylation of the sample prevented one yttrium 90 complex from forming. This same complex was absent in extracts from incubated soil.

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RH 00124 (Continued)

Uptake of radioisotopes found in fallout from contaminated soil organic matter by plant roots was measured and indicated that organic matter complexes restricted uptake in the order:

iron 59 $\gg$ ruthenium 106 $>$ strontium 90 $>$ cesium 137 $>$ iodine 131.

Four soil organisms were isolated which produced metabolites that complexed strontium 90 and cerium 144. The metabolites are produced in sufficient concentrations to drastically reduce plant uptake of cerium 144 by barley and soy bean plants. Strontium accumulation by barley is also retarded by the metabolites, the main component of which appears to be citric acid.

Soil organic matter has been found to be more reactive than inorganic soil colloids in complexing fission products such as cerium 144. Radiocolloid formation, the soil pH and salt concentration, and adsorption of organic matter on inorganic soil colloids affect the plant availability of Ce 144. These factors are currently being assessed.

Significance of Research: All soils have an ion retention capability. In an average soil 50 percent or more of this capability is due to the organic matter fraction. Since organic matter is quite reactive and concentrated in the surface layer of soil its reactions with fission products are of primary concern in evaluating methods for dealing with fission product contamination of soil. Organic matter complexes may play a key role in preventing fission products from entering the food chain by rendering these radioisotopes unavailable to plants and by accelerating their downward movement through soils

Publications: No additional publications.

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(1) RH 211-06	ALTERED METABOLISM STUDIES OF I-131 AND SR-90	JOHN E. CHRISTIAN, PH.D. DEPT. OF BIONUCLEONICS PURDUE UNIVERSITY LAFAYETTE, INDIANA

Research Objectives: The primary objective of this research was to study basic mechanisms by which (1) the absorption and utilization of the radionuclides strontium-90 and iodine-131 may be diminished, (2) the rate of elimination may be increased, and/or (3) the route of elimination may be altered with the aim of developing a practical means for preventing or lessening the contamination of food products derived from animals.

The longer term goal was the elucidation of basic information which will dictate and which can be applied to the development of practical methods for altering the absorption, concentration, excretion, and/or metabolism of fallout radionuclides in animal species including man. Basic principals found for the radionuclides strontium-90 and iodine-131 should be applicable (with modifications) to other radionuclides and thus be applied to the control of movement through the food chain.

Progress Report: The effect of sodium chloride administration on iodine-131 retention was studied in dairy calves. They ranged in body weight from 46 to 57 kg and in age from 26 to 53 days. A single oral dose of 10 g sodium chloride in aqueous solution was given immediately before the iodine-131 administration. Whole body radioactivity measurements were conducted in a scintillation counter. It was found that iodine-131 retention was markedly decreased by the sodium chloride administration. Retention was even further decreased when carrier iodine was given simultaneously.

A study was made of the effect of four dietary levels of stable iodine on whole body iodine-131 retention by calves. The calves were administered a single oral dose of iodine-131 12 days after the initiation of the daily stable iodine treatments. Each

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increase of stable iodine (0, 50, 500, or 1000 mg daily) caused a significant linear decrease in iodine-131 body retention when measured at 4 and 7 days post dosing.

The effect of several treatments on the whole body retention and milk secretion of iodine-131 in dairy goats was studied. These treatments were the administration of sodium chloride, potassium perchlorate, or potassium perchlorate plus potassium iodide. All treatments decreased both the whole body retention of iodine-131 and the secretion of iodine-131 into milk.

The effects of potassium iodide, potassium perchlorate, and pilocarpine nitrate on iodine-131 deposition in the eggs of laying hens were studied over a 16-day period. Potassium perchlorate caused a 91% decrease in iodine-131 deposition in the whole egg. A 68% decrease was obtained with potassium iodide. Pilocarpine nitrate did not significantly alter deposition.

A study of iodine-131 uptake in chicken ova showed that the uptake from a single dose is rapid and is nearly complete within a few hours. Autoradiographic studies of ova from hens receiving multiple doses of iodine-131 showed that it is deposited in the ova in an annular manner, with each concentric ring corresponding to a single dose.

A study involving modification of radiostrontium metabolism in the mammal is currently being done. The initial step involves a screening program to test the effectiveness of several chemical agents in increasing the mobilization of strontium in the bones of the rat.

Significance of Research: The results of this research may very well point the way for practical methods of preventing or significantly reducing the absorption and retention and/or promoting excretion of radionuclides in food producing animals and in man. This approach may yield methods of reducing absorption or altering the route of elimination in dairy cattle to reduce milk contamination. In addition, the data may indicate the

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feasibility of using stable iodine in children or as an additive to milk as a means of significantly lowering the iodine-131 uptake by the thyroid gland and thus lessening materially the hazard of drinking iodine-131 contaminated milk. These studies are considered important to the health and welfare of a population which may more increasingly be exposed to radionuclide contamination in foods and air from fallout on a broad area basis and from potential nuclear reactor incidents on a more localized scale.

Publications: No additional publications.

(1)

RH 00322-04

SORPTION OF HEAVY METAL RADIONUCLIDES BY MINERALS

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PITTSBURGH, PENNSYLVANIA

Research Objectives: The aim of this investigation is to study the chemical and physical state of plutonium as it might exist when released into treated municipal waters and lakes and rivers; also its interaction with particulate minerals. Both the equilibria and the rate of sorption and desorption of plutonium onto such surfaces will be studied. The elucidation of these phenomena will contribute information that will assist in the prediction of the fate of plutonium that accidentally or otherwise might contaminate a watershed.

Progress Report: The most prevalent oxidation state of plutonium likely to be encountered in natural waters is Pu (IV). At pH values above unity, the latter hydrolyzes appreciably to form such species as $Pu(OH)^{+3}$, $Pu(OH)_2^{+2}$ and $Pu(OH)_3^{+}$. Perhaps more important, however, is the formation of $Pu(OH)_4$ which is quite insoluble. The monomeric species of $Pu(OH)_4$ also polymerizes in the pH ranges encountered in natural waters, the monomers being joined by oxygen or hydroxyl bridges. Colloidal and larger size particles of both the $Pu(OH)_4$ precipitate and the polymer aggregates will be encountered in natural waters. Indeed, it is difficult to distinguish between the two and the term "Pu (IV) aggregates" may serve to encompass both species.

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Pu (IV) is also known to form complexes with such naturally-occurring anions as carbonate, sulfate, chloride and nitrate. However, except possibly for carbonate, the formation constants of these complexes and the ligand concentrations encountered in natural waters are generally not sufficiently large to form significant amounts of the complex and prevent the formation of Pu (IV) aggregates.

Other oxidation states of plutonium likely to be encountered in aqueous solution include Pu (III), Pu (V), and Pu (VI). Pu (IV) is known to disproportionate into these other oxidation states. However, in natural waters Pu (IV) will generally predominate.

Both filtration and centrifugation has been used in this research to characterize the plutonium solutions as to the distribution of sizes among the Pu (IV) aggregates and their rate of formation. For example, using filters of varying porosities at pH 7 a Pu (IV) solution aged for four days was shown to contain approximately 90 per cent of plutonium aggregates above one micron in size.

In the present research, in contrast to the filtration analyses, it has been shown that the mean particle size of Pu (IV) aggregates in the pH range encountered in natural waters is in the range of one micron, based on centrifugation experiments. Estimates of the molecular weight of the polymer by others are 4000 to 20,000,000. In any event, the present research indicates that the Pu (IV) aggregates are readily centrifuged. For example, in one experiment approximately 30 percent was centrifuged from solution at 100 G's in six minutes. In another, 90 percent was removed at 30,000 G's in six minutes.

The age of the solution greatly influences the size distribution of the Pu (IV) aggregates as shown by filtration through sintered silica discs of varying pore size and centrifugation analysis. This size distribution has a very great effect on the sorption characteristics of the plutonium. In experiments in which the plutonium solution was aged for up to 16 days prior to sorption, such solutions aged

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for five days gave subsequently higher rates of sorption than solutions aged for shorter or longer times.

It was also found that the sorption per unit surface area was greater onto larger diameter silica particles. Although additional research on the effect of particle size is required, this would seem to indicate that most of the sorbing plutonium would be associated with heavier silica particles which could settle out, rather than being carried along in a stream.

The sorption process onto silica was studied over the pH range of 3 to 9. The rate of sorption is generally show, even with agitation of the suspension. For example, at a pH of approximately 7 the rate of sorption had not leveled off until five to nine days. A possible rate limiting step in the sorption process is plutonium diffusion across the unstirred liquid film at the mineral-solution interface.

Since the age of the plutonium solution affects both the Pu (IV) aggregates size distribution, as well as the rate of sorption, this is evidence of the likely effect of aggregate size on rate of sorption, an additional correlation with diffusion as a rate limiting mechanism. However, another possible mechanism of sorption is polymerization or aggregate growth of plutonium already sorbed onto silica. Evidence for this mechanism is the general increase of the rate and amount of plutonium sorption with increasing amount of pre-sorbed material.

The pH of the ambient plutonium solution, both prior to and during the sorption process, is important in relation to both the rate and the amount of sorption. The rate and amount of sorption in the pH range of 7 to 9 is considerable higher than that in the range of 3 to 5. The charge and extent of ionization of the silica surface, the size and charge of the Pu (IV) aggregates, and the equilibria distribution among plutonium oxidation states are all affected by pH and are factors in the sorption process.

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The pH of the solution is also important in determining the rate of desorption. The rate into a slightly acid (pH 5) solution is considerably greater than into a neutral or slightly basic solution. The rate of desorption of Pu (IV) from silica is quite rapid at first as the loosely sorbed material comes off; this constitutes about one half of the sorbed plutonium and most of it desorbs in several hours. The more tightly bound plutonium requires many months for removal.

Significance of Research: With the increase in the production and processing of plutonium, the possibility of its accidentally contaminating water supplies and natural waters is increasing. In order to predict the fate and movement of this highly toxic material in such waters, it is necessary to understand its physical and chemical behavior, as well as its interactions with other materials present, such as mineral particulates.

To that end this research has been oriented towards studying those environmentally important variables that could effect this behavior, such as pH, temperature, ionic strength, and the aging of the colloidal plutonium system. In particular, the analysis of the distribution of the sizes of the colloidal plutonium and its rates of sorption and desorption onto silica (quartz) provide information necessary to understand and predict its movement through a watershed.

Publications: None

(1) RH 00354-03 METABOLISM OF RADIOSTRONTIUM

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TEXAS A&M UNIVERSITY
COLLEGE STATION, TEXAS

Research Objectives: The purpose of this project was to determine the metabolic route of radiostrontium and the possibility of increasing the elimination rate of radio

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strontium from contaminated living biological tissues by the alternation of the mineral content to the diet and by the addition of various additives to the diet.

Progress Report: In order to determine extremely low levels of radiostrontium in biological tissues, a method was devised which would give reproducible results with high efficiency and maximum sensitivity for low level counting of radiostrontium.

In order to calculate the vast amount of data which results from isotope studies such as these, a program has been written for the IBM 7094 computer which will calculate the absolute activity of β-emitters as tracers in biological studies involving liquid scintillation spectroscopy. The program provides rapid calculation of large amounts of data in extremely short periods of time. Decay, background counts, quenching, and dilution factors are all taken into account by the program. Precision and counting statistics are also provided in output data. In addition, the specific activity of any sample may be determined and data can be retrieved in any desired sequence.

Radiostrontium, recovered in the excreta of birds that had been fed phosphorous at various levels, was found to be highest in the group receiving the lowest phosphorus level. The birds receiving 0.1% phosphorus, excreted 81% of the injected dose of radiostrontium in 12 days; whereas, the group receiving 2.0% phosphorus excreted only 54% of the injected dose.

Data was collected from male chicks injected with Sr^{89} and fed diets containing 1.0, 2.0 and 3.0% calcium. Four bones and twelve soft tissues were taken at 2, 4, 8 and 12 weeks post injection time. The calculation of "elimination constants" (λ) from these data indicates that the elimination of Sr^{89} from the bone can be accelerated by a factor of two if the dietary calcium level is increased from 1.0 to 3.0%. Of the soft tissues taken for analysis, only the brain and eyes showed appreciable activity. The elimination of Sr^{89} from the brain is only about one-half as rapid as from bone tissue, but elimination of radiostrontium from the brain can also be doubled by an increase in dietary calcium from 1.0 to 3.0%. The elimination of Sr^{89} from the eyes

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is slow, being about one-fourth the rate as from bone tissue.

Additional investigations were undertaken in which chicks were fed various dietary levels of either untreated or activated verxite, a high-purity hydrobitite. It was found that this chelating agent was not effective in preventing intestinal absorption and skeletal deposition of Sr^{89}. A major problem involved in the use of cation binding agents in removal of strontium from tissue is that calcium is removed concurrently and often to a greater degree. Attempts have been made to use sodium alginate, a mucopolysaccharide from brown algae, and Pacific kelp as agents for the selected inhibition of the absorption of radiostrontium from the gut.

The smallest amount of Sr^{89} was recovered in the tibiotarsus of birds receiving 0.0% 0.5% dietary sodium alginate. However, when alginate was included in the diet at the 1.0% or 2.0% levels, the deposition of Sr^{89} increased. Both Sr^{89} deposition in bone and Sr^{89} excretion in groups receiving 1.0% and 2.0% dietary alginate were essentially the same. The greatest amount of the total dose of Sr^{89} recovered from the tibiotarsus was from birds receiving the Pacific dried kelp. The amount actually recovered in the bones of the group receiving the kelp was more than 1.5 times as high as the amount observed in the bones of the group fed an equivalent amount of alginate.

While the skeletal deposition of radiostrontium was increased by the addition of sodium alginate to the diet, the reverse was found to be true of Ca^{45}. Birds receiving no dietary alginate incorporated more Ca^{45} into the tibiotarsus than did any of the groups which received the alginate. In all groups, less Sr^{89} than Ca^{45} was excreted. The above data indicates that chelating type agents such as sodium alginate and exfoliated hydrobiotites are not effective agents in the gastrointestinal tract in discriminating against strontium and calcium absorption.

Serum from hens which had been given 25 μc Sr^{85} was separated on Sephadex G-25 column and found to contain Sr^{85} activity only in the non-protein fractions. On the other hand, the serum of a control hen showed the calcium to be present in both protein and

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non-protein fractions. These data suggest that, unlike calcium, strontium is not bound to proteins in the blood.

In an additional study, fifteen, seven-month-old laying hens were distributed at random into five groups of three birds each, placed in individual laying cages and fed a standard laying diet (Texas A&M University Poultry Research Center) that conatined 3.85% calcium and 0.45% inorganic phosphorus. The hens in each group were injected subsutaneously with 7.9, 27.0, 48.8, 175.3 μc Sr^{89} (as $Sr^{89}Cl_2$), respectively. The final group was not injected with Sr^{89} and served as the control. All hens were inseminated twice weekly with pooled semen from mature males. Feed and water were supplied ad libitum. Eggs were collected daily, stored at 55°F, and set at seven day intervals. Infertile eggs or dead embryos were removed, and Sr^{89} content was determined as previously described. Developing embryos were removed from the shell and separated from the extra-embryonic complex (EEC), i.e. the amniotic sac, chorioallantois and yolk sac starting at six days of incubation and the Sr^{89} content of each component was determined. Activity data were computed and extrapolated to time of injection in order that these data could be applied, for all practical purposes, to long half-life isotopes of each portion of the egg. A total of 1,233 samples was estimated for Sr^{89} content during the 62 day experimental period. The percent recovery of Sr^{89} in each component was found to be independent of dose.

An increased rate of disappearance of Sr^{89} activity from the egg shell was observed at about eight days incubation which corresponds with the beginning of calcification of the osteoid sheath. The Sr^{89} content of the embryo also began to increase more rapidly at this time and continued to increase at a fairly constant rate through the 12 days of incubation, which corresponds with the time the bone is being deposited in the form of trabeculae. At the same time the activity in the shell decreased in a similar manner. By the twelfth day of incubation the rate of uptake of activity in the embryo had doubled from 12 to 14 days of incubation and doubled again from 14 to 18 days. Correspondingly, the disappearance of Sr^{89} from the shell continued at a rapid rate from 12 to 20 days incubation.

STUDIES OF PROTECTIVE ACTION IN ENVIRONMENTAL CONTAMINATION

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

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The distribution of Sr^{89} in unincubated eggs among the shell, yolk, and albumen was found to be an average of 92.3%, 5.99% and 2.54%, respectively. Most of the variation was due to the yolk content which showed maximum activity at about 10-15 days following administration of the isotope to the hens. Otherwise, the distribution remained very constant and was independent of the dose. By six days incubation the overall distribution among the shell, yolk, and albumen was essentially the same.

As was noted above, the percent transfer of Sr^{89} from the shell to the embryo was dose independent, but the reproductive performance was influenced by dose. Egg production was reduced considerably as the Sr^{89} dose was increased and some birds ceased to lay 14 days after injection of the two highest levels of Sr^{89}. Fertility was not severly affected by high Sr^{89} levels, but the hatch of fertile eggs declined considerably at dose levels of 27μc or higher. Experimental design did not facilitate an adequate appraisal of embryonic mortality peaks. Teratological developments did not appear with sufficient frequency to justify drawing conclusions on radiation damage per se.

Significance of Research: A method developed from this research now enables us to count relative low levels of radiostrontium in biological tissues. A computer program has been developed which greatly facilitates the calculation of data on radioactive fallout contamination of biological materials.

The results obtained indicate that muscle tissue and other edible portions, not including bone, do not store radiostrontium to any extent, and that the radiostronium levels of these tissues reach a maximum level of activity shortly after exposure to the isotopes. It is also possible to increase the excretion of radiostrontium that has been deposited in the bone tissue by twofold if the calcium in the diet is increased from 1.00 to 3.00%. Chelating agents have been shown to have little effect on removing bone deposited radiostrontium. The bone deposited radiostrontium in laying hens can be transferred from the bone of the dam to the egg shell, and consequently, into the developing embryo. This has been followed through three consecutive generations with a decisive decrease of radiostrontium transferred from generation to generation. No

STUDIES OF PROTECTIVE ACTION IN ENVIRONMENTAL CONTAMINATION

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RH 00354 (Continued) radiostrontium has been found to be bound with any proteins in the blood; however, we have demonstrated that strontium can replace calcium in the blood clotting mechanism.

Publications:

Creger, C. R. and Colvin, L. B. "The Transference of ^{89}Sr from the Shell to the Developing Chick Embryo." Radiation Research, Vol. 32, No. 1, September 1967.

Colvin, L. B., Creger, C. R., Ansari, N. A., Allen, W. S., Couch, J. R. "Dietary Inorganic Phosphourus Effects on Retention and Deposition of Sr^{89}." Poultry Science, Vol. XLVI, No. 4, July 1967.

HUMAN EPIDEMIOLOGY STUDIES AND POPULATION EXPOSURE

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00039-07	FOLLOW-UP STUDY OF PATIENTS WHO RECEIVED THOROTRAST AND CONTROL GROUP	JORGE DA SILVA HORTA, M.D. INSTITUTO DE ANATOMIA PATOLOGICA FACULDADE DE MEDICINA LISBOA, PORTUGAL

Research Objectives: The aim of this project is to ascertain the possible pathological consequences of a radioactive drug ("Thorotrast" - a thorium dioxide colloidal suspension) used for radiological purposes in humans. Since this drug is retained practically indefinitely in the body, the Thorotrast population constitutes an invaluable source of material for study of the effects of long-term in vivo radiation in man.

Progress Report: In Portugal, over two thousand people received "Thorotrast" - a radioactive substance used as a contrast medium for radiological purposes. In some cases "Thorotrast" was given locally, and remained in place (loci of abscesses, for example), or it was completely evacuated afterwards (visualization of perinasal sinuses). But in the great majority of cases, "Thorotrast" had been administered systemically, remaining permanently in the spleen, liver, bone marrow and lymph nodes. We study these patients clinically, epidemiologically and pathologically.

We have tried to trace the patients who received the drug, (most of them had been injected more than 30 years ago and changed addresses many times), and many could not be found. When a patient was traced (by our social workers) he was then visited by a doctor of our study group who wrote the respective clinical and epidemiological file. Since then, the patients have been regularly visited - usually once a year - by a member of our study group, and all relevant data concerning the patient were reported on his file.

These clinical examinations include certain laboratory tests and radiographies. Some patients were even subjected to radiation measurements (dosimetry) and a study of chromosome aberrations was started.

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When it was possible (biopsy or autopsy), pathological studies were done. The alterations caused by "Thorotrast" in the different organs were studied and described. Among the pathological conditions found were fibrosis of the liver and spleen, malignant tumors of the liver, such as haemangioendotheliomata (considered "Thorotrast specific"), cholangiocarcinomata and leukemias. The number of malignant tumors and leukemias found in the "Thorotrast" patients have been shown by a statistical analysis to be far above those in untreated populations.

The laboratory tests in "Thorotrast" patients show also some specific and significant results. An article is being prepared for publication on these results.

Significance of Research: Since "Thorotrast" is a radioactive substance, we believe that the hazards caused in humans are mainly due to this radioactivity. Because we have access to a significant number of patients who have received the drug, it is important to do a complete study, in order to discover what are the consequences of radiation in human tissues and their prevention. The results of our studies will have extrapolation value for other internal emitters and radiation sources on humans in medicine. To quote a paper by Dr. P. Fischer and co-workers, the study of living Thorotrast patients may well contribute "toward the understanding of the mechanism of carcinogenesis in persons administered thorium dioxide".

A control group of patients who received another (non-radioactive) radiological contrast medium is also being investigated at the same time. Since this study is recent, the number of cases is not yet sufficient to give significant results.

Publications:

da Silva Horta, J. "Late Effects of Thorotrast on the Liver and Spleen and Their Efferent Lymph Nodes." Annals of the New York Academy of Sciences, Vol. 145, Art. 3, Pages 676-699, December 11, 1967.

HUMAN EPIDEMIOLOGY STUDIES AND POPULATION EXPOSURE

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00210-05	NEOPLASMS IN CHILDREN TREATED WITH X-RAYS	Louis H. Hempelmann, M.D. Dept. of Radiology University of Rochester Rochester, New York

Research Objectives: The objective of this research project is to learn all we can about induction of cancer and benign tumors in man by radiation exposure in a continuing study of 4,872 persons; these people have a history of receiving radiation therapy, usually in childhood. As controls, 7,752 non-irradiated siblings of the irradiated subjects are used. In particular, we are interested in studying the mechanisms by which irradiation of the thyroid induces cancer and in discovering the best means of treating and/or preventing this disease.

Progress Report: 1. A report published in the March 1967 issue of the Journal of the National Cancer Institute documents the results of the third survey in 10 years (1953-1963) of the incidence of neoplasms in a series of 2,878 irradiated young adults; these individuals were treated with X-rays in infancy for enlargement of the thymus gland. The incidence of cancer of all types in the irradiated population was four times that expected to occur spontaneously in the comparable group of adults in the general population while that in the non-irradiated siblings was not increased. Malignant and benign thyroid tumors, benign bone tumors (osteochondromata), leukemia and probably salivary gland tumors were present in excessive numbers in the irradiated population. If the incidence of pooled malignant and benign thyroid tumors was plotted against the estimated X-ray dose to the thyroid gland, a distinct dose response was observed; a substantial number of tumors arose in glands which received doses in the 100-400 rad range. Similarly, a dose response was also noted when the incidence of pooled types arising outside the thyroid gland was plotted against the cumulative air dose; most extrathyroid tumors occurred with air doses above 200 R (air). The incidence of thyroid neoplasms rose abruptly in the 10-19 year age period. The curve of incidence of thyroid adenomas versus age seems to flatten in persons in their twenties and that for thyroid cancer actually seems to decline after age 20. No cases of leukemia or bone tumors

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occurred in persons over twenty. One subgroup of 268 persons treated by a single radiologic technic was shown to have a particularly high risk of developing thyroid and other neoplasms.

Analysis of the data revealed that, although the incidence of thyroid neoplasms was dose-dependent, radiation exposure was not the only factor involved in the neoplastic transformation of the gland. The pathogenesis of radiation-induced thyroid neoplasms in man was found to be compatible with the multistage theory of carcinogenesis. Radiation-induced thyroid cell damage was the primary event (see 4, below) and thyroid stimulation during adolescence, probably the secondard or promoting event. Further analysis showed that the risk of developing thyroid cancer in the exposed population was 2.5 cases per million people irradiated in infancy per rad thyroid dose per year. The mail survey will be repeated in 1968.

2. Since it was obvious that the mail survey technic did not detect small or inconspicuous neoplasms, particularly those in the thyroid gland, it was decided to examine subjects in the high risk subgroup, mentioned above, still residing in the Rochester area. Of 105 persons examined, all of whom reported no thyroid disease on the mail questionnaire, 31 persons proved to have palpable thyroid nodules; 116 matched non-irradiated controls had only two small thyroid nodular lesions. Surgery was recommended in seven patients whose glandular lesions were suspected of being malignant; histological examination of the surgically removed tissues, however, showed only one case of cancer. All other cases were considered to be benign. Analysis of the data showed a strong dose response in the case of the incidence of thyroid nodularity but no demonstrable age response, i.e. no increase in incidence with increasing age. This supports the impression, previously mentioned, that the incidence curve of radiation-induced benign thyroid neoplasms flattens in subjects over 20 years. The report of this study has been published in the Annals of Internal Medicine, June 1967.

3. One hundred ten subjects in the high risk subgroup have been followed for four years. In two cases, sizable thyroid nodules regressed spontaneously and in two

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persons with a single thyroid nodule on the initial examination, subsequently developed a multinodular goiter. Sixteen persons with thyroid nodular disease believed to be benign were treated medically with daily doses of thyroid hormone. Solitary nodules responded promptly to this form of treatment but multinodular lesions were more resistant to therapy. The results of this study have been submitted to the Annals of Internal Medicine.

4. In a study published in Science, April 12, 1968, a plot of incidence of palpable thyroid nodularity in persons irradiated as children versus estimated cumulative thyroid dose shows a linear dose response between 20 and 1225 rads with no threshold or at least a threshold below 20 rads.

5. Thyroid cells removed surgically from two patients suspected of having cancer were grown in tissue culture. In one 28 year old women treated with 800 R in infancy, 36 percent of the cultured cells from non-tumorous thyroid tissue showed chromosomal aberrations; these aberrations are undoubtedly the result of the previous radiation exposure. In a 31 year old man treated with 400 R in infancy, 46 percent of non-neoplastic cells showed aberrations. In each case, the neoplastic cells showed fewer aberrations than the non-neoplastic cells. Persistant chromosomal damage in irradiated thyroid cells is undoubtedly the primary event in the development of subsequent neoplastic disease (see 1, above).

6. Other persons treated with X-rays in childhood for lymphoid hyperplasia of the nasopharynx are being examined if they are suspected of having a high risk of developing thyroid neoplasms. Particular emphasis is being given to a study of pituitary function and size in persons given head irradiation in childhood. Some of these individuals were treated with X-rays doses up to 3000 R (air). Since their pituitary glands were in the primary X-ray beam, the pituitary dose must have been substantial.

The names of 998 persons given X-ray treatment to the head were matched with names in the psychiatric registry for Monroe County, New York. Unlike the results reported by

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Dr. Roy Albert in persons treated with X-rays for tinea capita, the incidence of psychiatric illness in our irradiated series does not seem to be increased.

7. In a preliminary study of 606 women given X-ray treatments to the breast for post partum mastitis, 13 cases of breast cancer were identified by mail survey rather than the six expected to occur in a group of this size. In the five of these thirteen women given unilateral X-ray treatments only, the cancer always arose in the treated breast. Twenty benign breast tumors had been removed surgically rather than the four to five expected.

Significance of Research: The significance of this research lies in the fact that it provides quantitative data on the risks in man of developing cancer and other neoplastic disease after radiation exposure. Information regarding the clinical characteristics of radiation-induced tumors is also of importance. In the case of thyroid neoplasms, evidence is presented as to the mechanisms by which neoplasms develop after irradiation and as to best methods of treating radiation-induced thyroid neoplasms. All of these findings are of value with regard to the understanding and treating of the ever increasing number of cases of radiation-induced malignant disease in man.

Publications:

Pifer, J. W., Hempelmann, L. H., Dodge, H. J. and Hodges, II, F. J. "Neoplasms in the Ann Arbor Series of Thymus-Irradiated Children; A Second Survey." The American Journal of Roentgenology, Vol. 103, No. 1, May 1968.

Hempelmann, L. H. "Risk of Thyroid Neoplasms after Irradiation in Childhood." Science, Vol. 160, No. 1, pp. 159-163, April 12, 1968.

Pincus, R. A., Reichlin, S., Hempelmann, L. H. "Thyroid Abnormalities after Radiation Exposure in Infancy." Annals of Internal Medicine, Vol. 66, No. 6, June 1967.

HUMAN EPIDEMIOLOGY STUDIES AND POPULATION EXPOSURE

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
1) RH 00252-05	SR-90 IN DECIDUOUS TEETH IN THE GULF COAST AREA	ANTHONY DIMAGGIO, PH.D. DEPT. OF CHEMISTRY LOYOLA UNIVERSITY NEW ORLEANS, LOUISIANA

Research Objectives: The aim of this research project is to collect authenticated tooth samples from high population density locations in the States of Alabama, Florida, Louisiana, Mississippi and Texas. Strontium-90 content will be determined on teeth pooled according to tooth type, place of pre- and neonatal life, date of birth, and diet of mother. This data will be available for correlation between radioactive fallout and rainfall patterns, and for correlation between fallout and body burden.

Progress Report: An intensive publicity campaign coinciding with the opening of school in September was conducted in the target area over the four years, 1964-1967. Mailing containers with a printed questionnaire were distributed in the schools. As a result of the campaign approximately 15,000 deciduous teeth were collected, diagnosed and cataloged The catalog uses all 80 columns on an IBM card, storing approximately 40 bits of information. Auxiliary to the Sr-90 fallout study are 24 items of dental characteristics which form a wealth of raw data for basic dental research. From this collection of teeth approximately 150 samples were produced. Teeth from children of as similar a background as possible were pooled until a sample large enough to contain enough Sr-90 to assay was obtained. A chromatographic method of isolating Strontium in a lightweight chemical form was developed, modifying the methods of L. B. Farabee and of M. A. Wade and H. J. Seim. These lightweight samples absorb less radiation than the heavier samples obtained with other methods. This allowed us to "count" more radiation and therefore use as small a sample as possible. Small samples mean more samples per given number of teeth and lead to more statistical significance. Our data show that the amount of Sr-90 varies with rainfall, the highest amount of Sr-90 occurring in teeth from Eastern Louisiana-Mississippi Gulf Coast Area. The amount of Sr-90 in teeth also paralleled the amount of fallout generated from atmospheric testing. Our data concentrates on teeth from children born in 1957, 1958, 1959 and 1960, four years of increasing testing activity.

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Significance of Research: Assay of Sr-90 in deciduous teeth may well prove to be the most convenient and easiest way to monitor fallout and body burden of radionuclides. Our data will be correlated with bone Sr-90 content. Bone samples are, of course, more difficult to obtain. One must wait until proper autopsy material is available, a source currently not predictable. Construction of tooth and bone Sr-90 tables should result in simpler ways of calculating total body burden of radioactive materials. If ever necessary in the distant future, adult teeth may be removed for purposes of determining body burden in cases of accident, atomic warfare, etc. Many years must pass before the long range effects of the current body burden of Strontium-90 and other radioactive nuclides can be assessed. Our data will be there for this purpose.

Publications: None

RH 00298-04 FOLLOW-UP STUDY OF IRRADIATED TINEA CAPITIS CASES

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Research Objectives: The object of this study is to determine the long-term health experience of approximately 2,404 cases of tinea capitis (ringworm of the scalp) who were treated by X-ray epilation and 1,794 tinea capitis cases who did not receive X-ray therapy. Both groups were treated between 1940-1958 at the New York University Hospital Skin and Cancer Clinic. The purpose of the study is to characterize the nature and magnitude of chronic radiation injury due to X-ray epilation. The study was motivated by the current need in the field of radiological health protection for quantitative data on the relationship of radiation dose to chronic injury.

Progress Report: The study consists of three parts: (1) an evaluation of the medical experience of the irradiated and control populations by means of a health questionnaire, (2) a clinical and laboratory examination of a representative sample of the irradiated and control populations, and (3) a study of the X-ray dose to the various head structures associated with the Adamson-Keinbock technic.

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To date, about 80% of the cases have been located including 2,043 irradiated cases and 1,413 controls. The average age at the time of ringworm treatment was 7 years in both groups. The average elapsed time since treatment for both groups was 15 years. Fourteen confirmed cases of cancer, 5 of whom died, have been found in the irradiated group compared to one case (still surviving) in the controls. Cancers in the irradiated group included: 4 leukemias, 3 brain tumors, and 2 carcinomas of the scalp. The one cancer in the control group was a case of Hodgkin's disease. The incidence of confirmed psychiatric illness was considerably higher in the irradiated group (3.2%) compared to the controls (1.27%); the major differences between the two groups were in the incidence of personality disorders and psychoneurosis.

Direct physical examinations were carried out in 319 irradiated cases and 285 controls. The major finding was a 20% incidence of generalized loss of scalp hair. No evidence of radiation damage to the eyes or ears was observed.

Dosimetric studies indicated that the average scalp dose was about 800 rads. The dose to the brain ranged from 150 rads at the surface to 50 rads at the base. Doses to the eyes and ears and cranial marrow were 50, 70 and 435 rads, respectively.

Significance of Research: These data establish firm relationships between radiation exposure and (1) the tumorigenic response particularly of the scalp, brain and blood forming tissue in the skull, and (2) chronic tissue damage (or lack of it) in the eyes, ears and hair of the scalp. The results suggest the possibility that mental illness may be a manifestation of residual brain damage from ionizing radiation.

Publications:

Albert, R. E., Omran, A. R., Brauer, E. W., Dove, D. C., Cohen, N. C., Schmidt, H., Baumring, R., Morrill, S., Schulz, R. and Baer, R. L. "Follow-up Study of Patients Treated by X-Ray for Tinea Capitis." American Journal of Public Health, Vol. 56, No. 12, December 1966.

HUMAN EPIDEMIOLOGY STUDIES AND POPULATION EXPOSURE

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(2) RH 00303-03	PRENATAL EXPOSURE TO RADIOACTIVE IRON AFTER 15 YEARS	ROBERT M. HEYSSEL, M.D. DEPT. OF MEDICINE VANDERBILT UNIVERSITY NASHVILLE, TENNESSEE

Research Objectives: The short and long term hazards to man of externally delivered high dose ionizing radiation are well delineated. Knowledge of the hazards of low dose radiation or radiation exposure resulting from internally deposited radionuclides is much scantier. Additionally, there is relatively little information bearing on effects of radiation delivered in utero, particularly from internally deposited radionuclides. The population we have studied is made up of pregnant females and the resulting viable children who were exposed to radioactive ^{59}Fe during pregnancy in the years 1947 to 1949. The study was designed to detect deleterious late effects of radiation in the children.

Progress Report: Follow-up of 634 radioiron fed mothers and their infants and 655 control mothers and their infants is 95% complete. Analysis of the data indicate that three malignancies occurred in children born to mothers fed radioiron and one in control infants. Congenital malformations were no more frequent in the radioiron fed group than in controls. Of the malignancies, one was acute leukemia, one was lymphosarcoma, and a third tumor was a synovial sarcoma. Statistically, the differences between the number of malignancies which occurred and the number expected is not significant. Our conclusion is that we have been unable to detect an effect on induction of malignancies or congenital malformations due to the radiation exposure. It is recognized that the sample is small and that the findings are provocative with regard to malignancies. The manuscript describing the study is in late stages of preparation.

A manuscript is in press in the journal CANCER describing three brothers who died of hepatic cell carcinoma - an exceedingly rare occurrence of a rare tumor in a family. These cases were found during the follow-up procedures.

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Finally, one of the major problems in interpretation of the data relates to radiation dose. While originally the dose to the fetus was estimated at 5 to 15 rads, it is clear that the estimate has little basis in fact. At that time (1947), factors in the distributions of the radionuclide after oral ingestion during pregnancy were not recognized which render the actual amount of radioiron absorbed by the mother unknown. The amount going from mother to fetus at any particular stage of gestation is similarly unknown. This has stimulated our interest in iron metabolism in pregnancy. Our initial studies have been done in rats and a paper is in press in the American Journal of Physiology, entitled "Transfer of Iron Across the Placenta and Fetal Membranes in the Rat," by Dr. S. R. Glasser, Mr. Charles Wright, and Dr. R. M. Heyssel. These studies indicate little transport of iron from mother to fetus until the third trimester of pregnancy in the rat. At that time, however, as much as 50% of plasma iron goes to the multiple fetuses of the rat. Obviously dosimetry in the human also needs to consider trimester of feeding of the radioiron. We are embarking on studies of iron metabolism in human pregnancy using non-radioactive isotopes of iron and neutron activation analysis in order to study this further.

Significance of Research: This study is unique in that it attempts to assess the hazard to the human fetus of an internally deposited radionuclide. It has stimulated research on the tissue distribution of ^{59}Fe and the physiology of iron metabolism during pregnancy. As our knowledge is extended concerning the actual dose of radiation received by the fetus, we will be able to give quantitative information concerning the risk to the human fetus of certain low dose ranges of radiation exposure.

Publications:

Glasser, S. R., Wright, C. and Heyssel, R. M. "Transfer of Iron Across the Placenta and Fetal Membranes in the Rat." American Journal of Physiology.

HUMAN EPIDEMIOLOGY STUDIES AND POPULATION EXPOSURE

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00316-03	NATURAL RADIOACTIVITY IN HUMANS AND THEIR ENVIRONMENT	MASAHIKA OHIRA, M.D. DEPT. OF HYGIENE OKAYAMA UNIVERSITY MEDICAL SCHOOL OKAYAMA CITY, JAPAN

Research Objectives: This research studies the natural radioactivity and the effects of chronic exposure to the population in an area of high natural radioactivity in Japan. Environmental studies include the measurement of external radiation, radionuclides contents in water, soil, dust, and several foods in the test area compared with a control area. An epidemiological approach is planned to get some relationship to the environmental radioactivity.

Progress Report: The subject area includes uranium mines (Ningyotoge and other small ones in Okayama and Tottori Prefectures) belonging to Atomic Fuel Corporation and the Misasa hot spring containing a high quantity of radium and its daughters. The population of the area was roughly 70,000 in 1967.

a. External radioactivity has been measured at the ground surface, at the height of gonad (about 80 cm above the ground), and one meter above the ground. At the ground surface, the radioactivity measured was 12-25 microroentgen per hour in the test area, whereas 11-18 microroentgen per hour in the control area (Okayama City).

b. Levels of natural occurring radionuclides in the test area seem to be higher than those in the control area in almost all sampled materials.

The results in detail are as follows:

1. Radium (Ra) and Polonium-210 (Po-210) in Teeth. Average contents of Ra in the test area is 0.012 pCi/g wet weight of teeth whereas that in the control area is 0.009. Average of Po-210 is 0.057 pCi/g wet weight of teeth whereas that in the control area is 0.036.

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2. Polonium-210, Uranium (U) and Ra in urine. In Po-210 level studies, we can see the following decending order: uranium mine workers, test area residents, and lowest, in the control area residents. In U level studies, there might be the same tendency as Po-210. Due to the scanty amount of data, we can suggest nothing about Ra levels.

3. Po-210, Ra and U in Water. Po-210 in the test area seems to be higher than the control area. Ra and U are also suspected to be higher in the test area, though insufficient data prevents a clear conclusion.

4. Concerning the Foods. We have analysed foods and measured Ra content only. Average contents of 0.162 pCi/g ash (unit will be omitted hereafter) in rice, 0.053 in milk and 1.204 in eggs in the test area show higher value than the data in the control (0.063 in rice, 0.052 in milk and 0.370 in egg), but patterns of distribution do not ascertain any significant differences. On the other hand, vegetables such as egg plant, cucumber and potatoes show significant differences in averages, e.g., 0.236 (the test area): 0.033 (the control area) in egg plant, 0.501 : 0.008 in cucumber, and 0.354 : 0.086 in potatoes.

c. Epidemiological Surveys. The studies are now going on and any exact conclusion cannot be described yet. There is an indication that the test area has higher rates in infant mortality, infant deaths by congenital malformation and deaths by leukemia than the control area or whole Japan, but further statistical study will be needed before this can be positively established.

Significance of Research: The radioactivity of the test area was demonstrated to be not as high as had been expected before the investigation started and any positive conclusion cannot be deduced. In Japan, natural radioactivity has not been accurately analysed yet; especially the quality and quantity of natural nuclides in the environments are not ascertained yet. Accordingly, our investigation to make these things

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clear and to study the relationship between the environments and human health is worthwhile in itself even if the results would appear to be negative.

Publications:

Kurada, T. "Distribution of Naturally Occurring Radionuclides Belonging to Uranium Decay Series. Report 5. Po^{210} and Ra^{226} Concentrations in Drinking Water and Air." Medicine and Biology, 76(1): 34-37, January 10, 1968.

Kurada, T. "Report 4. Ra^{226} Content of Some Foods." Medicine and Biology, 76(1): 31-33, January 10, 1968.

Kuroda, T. "Report 3. Po^{210}, Ra^{226} and Uranium Contents in Human Urine." Medicine and Biology, 75(6): 234-236, December 10, 1967.

Kuroda, T. "Report 2. Pb^{210} and Ra^{226} Contents in Teeth of the Residents in an Area with Relatively High Radioactivity." Medicine and Biology, 75(6): 230-233, December 10, 1967.

Kuroda, T. "Report 1. Pb^{210} and Ra^{226} Contents in Human Teeth." Medicine and Biology, 75(6): 225-229, December 10, 1967.

RH 00461-03

TO COLLECT AND STUDY RADIOACTIVE NUCLIDES IN TEETH AND BONE

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Research Objectives: This study is designed to determine the accumulation of strontium-90 in fetal bone, deciduous teeth of children, and permanent teeth of young adults and to correlate this accumulation with rates of strontium-90 fallout as it appears in milk and diets. Although major emphasis centers around the St. Louis area, comparative data

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from other parts of the United States and the world are being gathered. Additional data on total strontium are also being obtained.

Progress Report: A. Deciduous Tooth Studies. The analysis of deciduous teeth have been completed for children born between 1948 through 1962, and partially completed through 1963. This comprises a time interval from periods of insignificant strontium-90 fallout until the years of maximum fallout that occurred during late 1963 and early 1964. The teeth of children born after 1963 are just beginning to be shed but these teeth will undoubtedly become available during the next two years. The data thus far obtained has led to an equation that adequately defines the relationship between diet strontium-90 and the amount of nuclide accumulating in the teeth. The equation(s) was developed by expansion of the basic equation of L. Z. Reiss:

C_T = X prenatal Sr^{90}/g Ca + Y postnatal Sr^{90}/g Ca (Eq 1) where C_T is the Sr^{90}/g Ca for the tooth crown and X and Y represent the fraction of tooth crown calcium deposited during pre and post-natal periods respectively. These factors vary with the kind of tooth and have been experimentally determined (Table 1).

Table 1

Fraction of tooth Ca deposited during the crown development

	Prenatal (X)	Postnatal (Y)
Incisors	0.32	0.68
Cuspids	0.06	0.94
First Molars	0.17	0.83
Second Molars	0.05	0.95

For children who were bottle fed from birth, equation 1 becomes:

$$C_T = (X)\ (1.2)\ C_d^m\ D_m + (Y)\ C_D^I\ D_I \qquad \text{Eq. 2.}$$

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where C_d^m is the mother's dietary intake of Sr^{90}/g Ca from milk and the total American diet is about 1.2 times the amount of Sr^{90}/g Ca found in milk, D_m is the discrimination factor against Sr^{90} between the mother's dietary intake and her fetus, C_D^I is the infant's commercial milk intake of Sr^{90}/g Ca and D_I is the infant's discrimination factor against Sr^{90} between dietary intake and bone.

In order to solve Eq 2, values for D_m and D_I must be experimentally determined. This factor appears to be 0.13 for D_m and 0.8 for D_I. Because the deciduous crowns calcify in less than one year, the Sr^{90}/g Ca in milk for the mother's diet (C_d^m) and the infant's diet (C_D^I) may be averaged for yearly intervals and with the discrimination estimates, equation 2 may be solved to become:

$$C_T = KC_d \qquad \text{Eq. 3}$$

where C_T and C_d are the Sr^{90}/g Ca for tooth crown and bottle milk respectively and K is a constant. The constant K has been determined to be 0.59 (incisors), 0.76 (cuspids), 0.69 (first molars) and 0.77 (second molars). Although the equations appear to be valid, continual refinement is required in order to establish the best values for discrimination factors, nuclide decay corrections, and dietary intakes. These refinements are minor and it is estimated that a 10-20% variation of any parameter will alter K by less than 5%.

Additional comparisons have been obtained for teeth of children who were breast-fed from birth versus those bottle-fed from birth, and between carious and sound teeth.

B. Permanent Tooth Studies. The basic study has been expanded to include the strontium-90 content of permanent first bicuspids that are removed for orthodontic purposes. Because these teeth do not begin to calcify until 2-3 years after birth, the maternal factor is removed from equation 1. Comparable treatment of the parameters results in an equation 4:

$$C_T = 0.31\ C_D \qquad \text{Eq. 4}$$

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where C_T and C_D are the strontium-90/g Ca for tooth crown (C_T) and diet (C_D). This equation is entirely analogous to that derived for deciduous teeth with appropriate modification for the time of development and discrimination factors.

C. Fetal Bone. Studies have been performed on fetal bone and tooth buds of fetuses aborted between 1961 and 1968, covering the period before and after peak fallout levels. These studies evaluate the pre-natal function of Equation 1 and describes the relationship between the mother and her fetus. The fetal studies modify two variables which were previously estimated. The first variable suggests that the mother's diet is better expressed as 1.6 times the dietary milk Sr^{90}/g Ca in accord with recent suggestions by the Federal Radiation Council. These studies, comparing the strontium-90 content of tooth buds, mandibular bone and femur bone, indicate that strontium-90 in mineralized fetal tissues are in equilibrium. Thus, equation 1 may be re-written as:

$$C_B = 1.6\ C^m_{d-6}\ D_M \qquad \text{Eq. 5}$$

where C_B is Sr^{90}/g Ca in fetal bone, C^m_{d-6} is commercial milk strontium-90/g Ca during the previous six month interval prior to birth or abortion and D_M is the discrimination factor. With 0.13 for D_M the final equation becomes equation 6:

$$C_B = 0.21\ C^m_{d-6} \qquad \text{Eq. 6}$$

D. Ancillary Studies.

a. During the tenure of this investigation and the availability of flame absorption spectrometry, it became desirable to measure the total strontium content of mineralized tissues, milk ash and food ash. These studies are in progress but sufficient data has not as yet accumulated.

b. A complete study of the strontium-90 content of teeth and bone of South American Howler Monkeys (Alouatta caraya) was undertaken with the collaboration of Dr. Rene Malinow of the Oregon Regional Primate Research Center.

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c. A complete strontium-90 balance study was performed in young rabbits following feeding of naturally contaminated rabbit chow.

Significance of Research: The research project described above defines the factors that relate the accumulation of strontium-90 into the teeth and bones of man and other animals with the dietary intake of the nuclide. On completion of the study, it will be possible to predict how much strontium-90 will accumulate in teeth and bone of children under any conditions of fallout. Furthermore, the data describe the amounts of strontium-90 that has accumulated in the teeth and bones of children which may be related to future radionuclide health hazards. It must be recognized that the ultimate effects of strontium-90 fallout on the health and welfare of human and animal populations may not be known for many years. Additionally, the study is of historical and scientific value by thoroughly documenting an artificially created, hazardous condition which would, hopefully, never occur again.

Publications:

Rosenthal, H. L. "Chronological Age Determination as Estimated from Strontium-90 Content of Teeth and Bone." Biology of the Howler Monkey (Alouatta caraya), Bibliotheca Primatologia, No. 7, 48-58, 1968.

Rosenthal, H. L., Austin, S. A., Gilster, J. E. and Bird, J. T. "Accumulation of Strontium-90 into Human Fetal Teeth and Bone." Proceedings of the Society for Experimental Biology and Medicine, Vol. 125, 493-495, 1967.

RH 00494-02

LATE EFFECTS OF THORIUM DIOXIDE RETENTION IN HUMANS

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Research Objectives: The objective of this study is to establish the morbidity and mortality experience of a large group of patients who received Thorotrast for cerebral

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arteriography. All living patients will be subjected to intensive physical and laboratory examinations to detect the sequelae of their long-term radiation.

Progress Report: To date, 190 of 195 patients who received Thorotrast at the Massachusetts General Hospital, and 124 out of 129 patients who received Thorotrast at the Lahey Clinic have been identified, located, and interviewed. Six malignant tumors have been detected in the MGH population, none of which is of a type known to be a result of long-term radiation. Eight tumors have been discovered in the Lahey Clinic population. Of these, one case has been of Hogkins Disease, developing two years post arteriography; and one case has been of chronic lymphocytic leukemia, developing 14 years post arteriography.

Approximately 40 of the MGH Thorotrast patients have been examined. Examination has included physical examination, total body radiography, whole-body counting, chromosome analysis, liver function tests, complete blood count, and morphological analysis, and a variety of other laboratory tests. Frequent abnormalities have been shown in the alkaline phosphatase, Bromsulphaline retention test, protein electrophoresis, and chromosome analysis. Radiographs have demonstrated Thorotrast deposited in the liver and spleen of all patients. Whole-body counting has revealed that the Thorotrast retained within the body is directly related to the amount injected, in the range studied.

Significance of Research: Human data on the effects of chronic low-dose internal emitters are very limited. Most information until this time has been provided by study of the radium dial painters. The Thorotrast population represents a unique opportunity to study a large group of patients who carry reasonably well-known body burdens. The effects on humans of chronic low-dose internally emitted radiation is vital for the establishment of exposure standards for industrial workers and for the general population to possible environmental contamination.

Publications: None

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RH 00021-13	THE EFFECTS OF X-IRRADIATION ON THE SMALL INTESTINE	JAMES W. OSBORNE, PH.D. RADIATION RESEARCH LABORATORY UNIVERSITY OF IOWA IOWA CITY, IOWA

Research Objectives: The broad objective of the research project is to learn more about the consequences of exposing the small intestine of rats to X-irradiation. A more specific objective related to work which has been in progress for some time is that of determining the sequence and quality of radiation-induced changes which precede the early loss of intestinal mucosa and in many cases the later appearance of intestinal tumors.

Progress Report: As a result of irradiating only the small intestine of rats under special conditions, it has been possible to induce two kinds of lesions in the small bowel. One may be regarded as adenocarcinoma and the other as a benign change associated with an inflammatory reaction and serosal fibrosis. Intestinal carcinoma is normally a rare occurrence in mammals but it was found that the exposure of only the small bowel (excluding the duodenum) of rats to 1400 R - 1600 R of X-radiation was one way to produce it. Another way was to X-irradiate the exteriorized gut with higher doses (1600 R to 2500 R) during a time when blood flow to the intestine was temporarily restricted. Later it was discovered that delivering part of the X-ray exposure of the gut during the blood restriction procedure followed immediately by exposure under natural conditions was even more effective in inducing the malignant neoplasm. Summing over all treatments, the tumor has been found in about forty percent of several hundred animals used. It has been found as early as a few months after exposure, but generally was not palpable in the abdomen for eight-ten months after a treatment.

Deaths in tumor-bearing rats were usually due to intestinal obstruction, but some succumbed owing to bowel perforation and peritonitis. The tumors varied in size (0.5 cm-3.0 cm in diameter) and usually only one was found. However, in some cases,

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several tumors of varying size were distributed along the small bowel, but they were rarely found any nearer the ileocecal junction than 10 cm. Upon examining histological sections of the tumor and normal-appearing adjacent intestinal tissue, it was felt that the criteria necessary for terming the lesion an adenocarcinoma were satisfied. Glandular epithelium was found in fibrous tissue and epithelial cell invasion of muscle and nerve could be demonstrated in a number of cases.

A benign lesion was caused in the intestine by X-irradiation of only small segments of gut. A number of combinations of X-ray exposure and segment size were used. As little as 1500 R and as much as 5000 R were employed while segment size was either 2.5 cm or 7.5 cm. When the intestine was exteriorized again a few days after the irradiation procedure, there was no difficulty in determining which area had been irradiated. The exposed area was very red and edematous; the gut wall appeared to have thickened but this may have been due only to edema. By the twentieth day, most animals had a lesion palpable from outside the body. Weeks to months later the animals died. Death could usually be ascribed to intestinal obstruction although in a few cases intestinal perforation occurred. At the earliest sacrifice date, six days, a clear line of demarcation between irradiated and unirradiated tissue could be seen. The intestinal mucosa was absent, there was hypertrophy and/or edema of the longitudinal muscle layer and the beginning of serosal fibrosis. In those animals where serosal fibrosis was prominent, the process progressed rapidly until by day 20, fibrous tissue was the dominant feature of the original irradiated area. Where exposures were 5000 R, the lost mucosal epithelium was not replaced during the 44 day observation period. With lower exposures, some epithelial cell regeneration began by day 8. By day 24, e.g., the degree of organization in villi tended to be normal in intestines from animals in the 3000 R - 7.5 cm group. But often the crypts were hyperplastic showing that cell proliferative activity above normal was occurring. The findings suggest that there is a certain level of X-ray exposure and intestine involvement above which satisfactory repair cannot occur. After irradiation of 2.5 cm segment with 5000 R it would be logical to expect recovery in the irradiated area by proliferation of cells from the adjacent unirradiated area. But this does not occur, perhaps because fibrosis is such a dominant feature and blocks such migration. Thus recovery never occurs because the

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exposed area itself is incapable of proliferative activity. A lower exposure is favorable for recovery, but irradiating a larger segment amplifies the problem of replacing the damaged mucosa in the center of the irradiated area within a time period before fibrosis becomes limiting or other problems arise.

Significance of Research: There are a number of situations in which a portion or the entire small intestine of man is exposed to radiation. This may occur in the course of a radiation therapy treatment to some other area or following the ingestion of radioisotopes. Since the intestine is so responsive to a radiation exposure and may eventually become malignant, it seems important to study carefully in animals the factors which can influence the processes of damage and repair of the small bowel.

Publications: No additional publications.

RH 00065-13

IONIZING RADIATION, BEHAVIORAL DEVELOPMENT, AND AGING

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Research Objectives: The aim of this research is to determine the behavioral effects of ionizing radiation administered during the developmental period. The effects of age at the time of exposure as well as dose are determined. Different types of functions, simple as well as complex, are investigated. Animals are studied throughout their life-span to see whether the effects of irradiation remain constant or whether they become exacerbated or ameliorated. The latter aspect of the research also should provide data for an analysis of behavioral changes during aging. The interrelationship of the various changes may provide us with a model for a behavioral embryology.

Progress Report: In the initial phases of our research, we attempted to determine the kinds of behavioral changes which result from irradiation in rats either pre-

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or neonatally. We found that these animals were deficient in maze learning, in several types of locomotor tests, in depth and olfactory discrimination, and in adjusting their food or water intake to deprivation schedules. They were hyperactive in novel situations; their heart rate increased more rapidly than that of control animals when presented with novel stimuli; and in general, they exhibited behaviors that are usually labeled fearfulness. The latter actually produces more rapid avoidance conditioning; the animal must learn either to do something in order to avoid an electric shock or it must inhibit doing something to avoid the shock.

In learning situations, the most pronounced differences occur early during training. With prolonged training, the performance of the irradiated animals may approximate control levels.

For locomotor deficits and maze learning, the lowest effective dose was around 25 R administered sometime during days 15 to 18 of gestation (in the rat the gestation period is 21 to 22 days). If irradiation occurred after about day 18 or neonatally, a larger dose was necessary before locomotor deficits could be observed. For the detection of hyperreactivity to novel stimuli, larger doses were required. Here the minimal effective dose was between 100 and 200 R.

The severity of the motor deficits is apparently relatively constant throughout the animal's life-span. We tested animals at various ages, and we could not detect any progressive changes in the deficits. Similarly, measuring aversive conditioning we obtained no evidence for any changes in the conditionability of irradiated animals during their life-span. Actually, in measures of activity in novel situations, it seemed that the difference between control and prenatally irradiated animals tended to decrease with increasing age. It would seem, that the normal decrement in arousal with increasing age tends to reduce the expression of the hyperreactivity which may be observed in young irradiated animals.

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In most studies of prenatally irradiated rats, the irradiated mother rears and takes care of her own pups. The possible influences of the maternal behavior of an irradiated mother on the young has heretofore not been investigated. We, therefore, measured the effect of an irradiated mother on the behavior of the pups which she mothered. We found that such pups, whether irradiated or control, tended to grow more slowly and they tended to be hyperactive. However, we also found that not all, or most, of the differences observed in other studies between irradiated and control rats could be ascribed to the maternal influence. Thus, results of previous studies in which irradiated pups were not fostered by control animals were not invalidated. Rather, the magnitude of the difference previously observed between control and irradiated pups in which the latter were not reared by control mothers was exaggerated by the contribution of the irradiated mother. Since most behavioral studies indicate that irradiation of an adult animal with sublethal doses has few long-lasting behavioral consequences, the present findings are somewhat unique. Here in pregnant females, a 200 R dose did result in subtle changes of maternal behavior.

Data were also obtained on normative age changes of behavior in rats. Beginning with young adulthood, speed of avoidance conditioning decreases; this change is, however, primarily a reflection of the change in the speed of response. If response speed is held constant then the rate of conditioning does not vary with age. Normative data were also obtained on changes in activity and exploration. Here, it was found that the change in activity in novel situations decreases with age as well as the amount of non-locomotor exploration as measured by the amount of sniffing. A satiation effect occurred more rapidly in older than in younger animals. In summary, the latter data would indicate that with increasing age there is not only a decrement in general activity in animals, but also a decrement in the reactivity of older animals to novel stimuli.

Significance of Research: The great vulnerability of the developing organism to ionizing radiation has been recognized almost since the discovery of X-rays. There are reports in the clinical literature of children who were irradiated in the womb

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and who later showed defective development. A large percentage of the abnormalities occurred in the nervous system. However, in other instances where individuals were similarly exposed, no gross abnormalities could be detected.

There are no systematic data which would delineate the kind of radiation-induced behavioral malfunctions which may result from irradiation during the developmental period. While there is substantial information about anatomical malformations, ultimately the significance of the structural changes must be assessed in terms of their functional consequences.

It is true that our research is with animals and that any extrapolations to the human level must be with caution. However, it is also obvious that in research on radiation hazards, we cannot experiment on the human level. Also, in an evaluation of human clinical cases on the behavioral level it is usually feasible only to detect gross defects, and it would take a very large population to detect subtle changes. Animal research where large numbers may be studied, may provide us with data on the lower limits of exposure levels which may result in significant deleterious behavioral changes.

Publications:

Furchtgott, E., Tacker, R. S. and Draper, D. O. "Open-field Behavior and Heart Rate in Prenatally X-Irradiated Rats." Teratology, Vol. 1, No. 2, May 1968.

Furchtgott, E. "Long-Term Behavioral Effects of Prenatal X-Irradiation." Abstract Ab-12, Radiation Research, Vol. 31, No. 3, July 1967.

Furchtgott, E. "Behavioural Effects Produced by Irradiation During the Developmental Period." American Zoologist, Vol. 7, No. 4, November 1967.

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(1) RH 00081-06	BASIC MECHANISMS IN THE INDUCTION OF CONGENITAL RADIATION CATARACTS	ROBERTS RUGH, M.A., PH.D. DEPT. OF RADIOLOGY COLUMBIA UNIVERSITY NEW YORK, NEW YORK

Research Objectives: To determine the short and long term effects of ionizing radiations and radio-mimetic agents on biological systems. Interest has been focused on the embryo and fetus (of the mouse) because (1) we are particularly well fitted for such studies, (2) there are very few centers where such studies are in progress, and (3) the rapidly proliferating cells of the embryo are highly radiosensitive.

Progress Report: We have shown that 100 R X-rays delivered to the mouse embryo at the time the egg is fertilized results in 90% of both males and females developing cataracts by 18 months of age. There are also other stages of gestation when exposure to X-rays will cause a statistically significant increase in cataracts but the highest incidence follows exposure prior to the first cleavage of the egg. This study is being carried further by an attempt to determine the threshold dose of cataractogenesis; the possible hereditary predisposition to its development; and the possible chromosomal corollary of such effects.

Another study, involved 34,508 mouse fetuses, each of which was examined, weighed, sexed, and the anomalies catalogued following low level exposures to X-rays (10, 15, 25, and 50 R) at hourly intervals from fertilization to 12 hours, then at 18, 24, and 30 hours post-conception to determine whether such low exposure levels might in any way affect development. It was found that embryos exposed at 7 hours post-conception were the most radiosensitive, and that exposures of 50 R had effects at all hours from 2 to 11 post-conception. There was no significant increase over the controls in the anomalies produced, but there was a reduction in litter size suggesting very early deaths and resorptions of the embryos even prior to the first cleavage time. This study has clinical significance since an exposure of 10 R is

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within the range of some diagnostic procedures, and a woman could well be exposed to such a dose prior to her realization of a very early pregnancy.

Current studies are directed toward: Immediate effects of sperm irradiation and the effects on subsequent generations: the possibility of low level fetal exposures and changes in adult LD/50/30 levels; the similarity or dissimilarity of effects of radio-mimetic agents (chlorambucil, mustargen, thiotepa, etc.) on the mammalian (mouse) embryo, establishing the threshold effect for the production of congenital anomalies.

Significance of Research: This research contributes to an understanding of the biological effects of ionizing radiations, but it is becoming clear that for the embryo and the gonads there may not be a threshold level, but rather that any radiation has its effect. Since it is often not possible to identify a pregnancy until four-five weeks post-conception, it is recommended that the pelvis of human female of reproductive age be not exposed to any radiation except during the first 9 days after the onset of menstruation in order to avoid the possibility of exposing an unrecognized pregnancy. It is now quite clear, based upon mouse studies, that the comparable age of the human embryo, from conception to about 6 weeks is particularly radiosensitive and that any radiation of high diagnostic level could conceivably cause anomalies. The threshold dose of 25 R has been suggested as one above which an exposed fetus should be aborted, particularly if exposed during the first trimester. This will surely be revised downward to the level of 10 R, especially when the exposure occurs during the first four weeks.

The emphasis of these studies has not been to establish hazard levels but it so happens that the embryo, fetus, and probably the germ cells are so highly radiosensitive that caution should be urged of all radiologists.

Publications: No additional publications

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RH 00140-05	PROTECTION AGAINST RADIATION DAMAGE TO CHROMOSOMES	SIDNEY MITTLER, PH.D. DEPT. OF BIOLOGICAL SCIENCES NORTHERN ILLINOIS UNIVERSITY DEKALB, ILLINOIS

Research Objectives: The objective of this research program is to obtain information on radiation-induced genetic damage and the biological repair system. Eventually, we may then be able to prevent or control the permanent injury to the chromosomes and genes. Currently ATP (adenosine triphosphate) and its role in aiding the repair of radiation-induced mutations and broken chromosomes is under study. Dominant lethal mutations, recessive sex-linked lethals, deletions of the X chromosomes, and translocations of chromosomes are induced by X-rays, and the effect of ATP injections in decreasing the amount of the genetic damage is determined.

Progress Report: At the start of the program, antimitotic chemicals were tested as possible protection against radiation-induced genetic damage. Urethane and colchicine which were reported to increase radio-resistance were used in an attempt to prevent the radiation-induced loss of a chromosome. Drosophila melanogaster was chosen as a test organism, for the genetics of this animal is well understood and methods have been developed which enable one to determine the various genetic radiation damages. A ring X-chromosome was utilized which was sensitive to breakage and the loss could easily be detected by the appearance of exceptional XO males. A method for injecting the chemicals was developed, and the treated males were exposed to 2000 R. By means of a daily mating schedule, the various stages in spermatogenesis that had been injured could be separated. Each brood then represented a stage in spermatogenesis: the first day brood was the result of mature spermatozoa, the second day brood represented immature spermatozoa at the time of irradiation, and the broods after day 9 represented spermatogonia. Thus the protective effect, if any, could be determined on particular cells in spermatogenesis.

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Urethane reduced the radiation-induced loss of the ring X or Y chromosome, as determined by the number of XO male offspring. Urethane alone increased the loss of the ring X chromosome primarily in the mature spermatozoa and older spermatids, whereas X-rays induced chromosome loss to a greater extent in those cells in or about meiosis. Pre-treatment or post-treatment with colchicine by means of injection near the testis gave an apparent protection against radiation-induced chromosome loss; but colchicine alone induced sterility by reducing the number of gametes. The colchine tended to eliminate those cells in spermatogenesis that were in or about meiosis at the time of treatment.

At present the research is involved with ATP (adenosine triphosphate); we have found that ATP does decrease the damage of radiation by aiding in the repair of broken chromosomes. Dominant lethals were induced in the offspring of those males that were treated with ATP before X-ray treatment. If the males were injected with ATP which acts as one of the main fuel components of the cell and then X-rayed and permitted to recover in an atmosphere of nitrogen, the ATP protective effect was eliminated. Evidently the ATP acts as a source of energy for the repair, but is more efficient if oxygen is present. ATP does not protect against recessive lethal mutations.

An attempt is being made at present to find a chemical that will stimulate ATP production and thus decrease radiation-induced genetic effects. The cations Mg^{++} (magnesium), Ca^{++} (calcium), and Mn^{++} (manganese) are being used in an attempt to decrease the amount of dominant lethals.

Significance of Research: Since we live in an era of Atomic Energy, the exposure of humans to radiation is ever increasing. One of the subtle effects of radiation is the induction of mutations and chromosome aberrations which can be passed on for many generations. If induction of mutations increases, the "genetic load" may reach such proportions that existence of mankind may be in danger. Sterility will increase along with numerous so-called "birth defects". An attempt is being made to understand the radiation-induced genetic damage and to discover methods by which this damage can

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be repaired by biological processes. At present we have found adenosine triphosphate (ATP) to be of some help in preventing dominant lethals. Although there have been some compounds discovered which will protect against death from radiation, at present we have no chemical that will protect against radiation induction of mutations.

Publications:
Mittler, S. and U, Raymond. "Adenosine Triphosphate: Protection against Radiation-Induced Chromosome Loss in Drosophila." Science, Vol. 152, No. 3725, 1087-1088, May 20, 1966.

RH 00218-06

β-IRRADIATION EFFECTS ON DERMAL VASCULAR PERMEABILITY

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Research Objectives: Studies are being conducted on both early and late increases in the permeability of blood vessels of β-irradiated skin. The efficacy of various anti-inflammatory drugs to suppress these vascular changes and the subsequent ulceration is being evaluated. Insight into the mechanism(s) responsible for these changes is expected by choosing drugs with different sites of action. It is hoped to arrive at a combination of drugs which will effectively suppress the permanent skin injury associated with radiodermatitis. In addition, attempts will be made to isolate and identify the factor(s) responsible for the radiation induced skin inflammation.

Progress Report: We originally observed postirradiation increases in the activity of several destructive epidermal enzymes (nucleases) which attack both cellular DNA and RNA. The enzymes responsible for this heightened activity may have originated from the blood due to an increase in permeability of skin blood vessels and, therefore, we studied the effect of β-irradiation on dermal vascular permeability. Utilizing

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circulating ^{125}I-labelled guinea pig serum, we demonstrated an early biphasic (3 and 18 hour) increase in permeability of blood vessels of β-irradiated guinea pig skin. This sensitive quantitative tracer method for measuring increases in leakage of blood vessels in skin was used to evaluate the efficacy of various drugs in suppressing the early increase in vascular permeability. The acute clinical signs of radiodermatitis, erythema, hyperemia, ulceration, and sloughing of epidermis were effectively suppressed by C1-583, indomethacin, promethazine HCl, and E-amino-n-caproic acid. Systemically administered C1-583 or indomethacin proved to be the most effective. We are presently evaluating the topical application of these and other drugs in suppression of acute radiation dermatitis. Thus far, triamcinolone has proven most effective.

During the course of our studies, it was also found that ^{125}I-labelled guinea pig serum albumin can be detected on the hair one hour post injection, reaching a level of three times background by six hours. This would suggest that measuring accumulation of radioactive isotopes in hair might prove to be a simple means of determining the amount of isotope (radioactive iodine, etc.) ingested by mammals. (Editor's note: See RH 00042-07) Lastly, extracts from irradiated epidermis have yielded two partially purified proteins with the ability to produce a sustained increase in the blood vessel permeability after injection into the skin. These may be the active permeability factors released after irradiation injury.

Significance of Research: To our knowledge, there has been no published systematic evaluation of the ability of anti-inflammatory drugs to suppress acute radiodermatitis. Our studies (in press) have shown that no one drug can effectively suppress the complex sequence of changes in skin resulting from a severe β-irradiation burn. Therefore, utilizing some of the more potent anti-inflammatory drugs both systemically and topically, we hope to come up with a combination of drugs which will effectively suppress the early increase in vascular permeability and the subsequent ulceration. Given both before or immediately after irradiation, this type of drug therapy will be of considerable aid to those concerned with permanent skin damage resulting from cancer radiation therapy, and it would also be useful after skin injury resulting

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from accidental irradiation or fallout. Further purification and characterization of the protein-type permeability factors which we have succeeded in isolating from irradiated skin should aid in the design of more effective agents for suppression of radiodermatitis.

Publications:

Song, C. W., Drescher, J. J. and McCarron, Jr., D. J. "Anti-Inflammatory Suppression of Radiodermatitis." 1968 Annual Meeting of the American Society for Experimental Pathology, Atlantic City, New Jersey, April 15-20, 1968.

Tabachnick, J. and Freed, R. M. "Increased Activity of Skin Surface Nucleases after Beta-Irradiation." 1968 Annual Meeting of the American Society for Experimental Pathology, Atlantic City, New Jersey, April 15-20, 1968.

Song, C. W. and Tabachnick J. "Epidermal Cell Population Kinetics in Albino Guinea-pig Skin." Nature, Vol. 217, No. 5129, February 1968.

Tabachnick, J., Song, C. W. and Drescher, J. J. "Effect of Anti-Inflammatory Agents on Radiation-Induced Skin Injuries." Abstract Fa-1, Radiation Research, Vol. 31, No. 3, July 1967.

Tabachnick, J., Perlish, J. S., Chang, L. F. and Freed, R. M. "Enzymatic Changes in Beta-Irradiated Epidermis of Guinea Pigs: Acid and Alkaline Phosphatases and Inorganic Pyrophosphatases." Radiation Research 32, 293-308, 1967.

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RH 00243-05	EFFECTS OF C-14 ON CYTOGENETICS	SHIELDS WARREN, M.D. CANCER RESEARCH INSTITUTE NEW ENGLAND DEACONESS HOSPITAL BOSTON, MASSACHUSETTS

Research Objectives: The research objective is to determine the effect, if any, of ^{14}C incorporated into DNA of mammalian tissues using the germinal epithelium of mice as a test object.

Progress Report: We have evolved a satisfactory chamber for the experimental exposure of mice (in numbers up to 16) to measured amounts of $^{14}CO_2$ permitting the use of a wide range of levels of concentration of ^{14}C in the air and hence in the DNA of cells of the mice as their tissues come into equilibrium with the level of ^{14}C in the inspired air. The experimental runs have been carried out to over 60 days. We have worked from the very low level of about 500 disintegrations per minute per liter of air due to the presence of ^{14}C to several orders of magnitude above that level. We measure the incorporation of ^{14}C in DNA by two techniques. One is chemical separation checked by radioautographic observation of the tissues. At very low levels minor variations in the ^{14}C content of DNA have been found which may relate to differences in metabolic activity of tissues but probably represent technical difficulties due to the very low level at which we are working. With higher concentrations of $^{14}CO_2$ satisfactory reproducibility of results is obtained. The other method is to check the presence of ^{14}C by radioautography.

In addition to these methods, with the aid of Dr. Masuo Kodani of the University of Missouri we are carrying out cytogenetic observations. Dr. Kodani is studying the cellular differences in testicular germinal epithelium. He finds at the higher levels of ^{14}C concentration an increase in polyploidy in some of the cells. Because of the danger of accidental exposure of $^{14}CO_2$ elaborate and time-consuming precautions have had to be undertaken in the interest of safety. Fortunately, these have functioned satisfactorily. Another troublesome aspect of engineering has been the problem of maintaining the animals in healthy condition during the many days of living in an inadequately controlled environment.

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Significance of the Research: A great deal of scientific speculation has gone on as to the significance of incorporation of ^{14}C into DNA of germinal epithelium. Virtually no experimental data exist and the present work is an attempt to determine what, if any, effects there are. This experiment is significant not only to obtaining data pertinent to the current established occupational permissible doses for ^{14}C but also to the potential effect, if any, of the gradually increasing amounts of ^{14}C in the biosphere.

Publications:

Kury, G., Rev-Kury, L. H. and Crosby, R. J. "The Effect of Selenous Acid on the Hematopoietic System of Chicken Embryos." Toxicology and Applied Pharmacology, Vol. 11, No. 3, November 1967.

Joftes, D. L. "^{14}C Content of Tissues of Mice After Prolonged Breathing of $^{14}CO_2$-Contaminated Air." Abstract, Radiation Research, Vol. 31, No. 3, 1967.

RH 00272-04

THE AGE FACTOR IN RADIATION SENSITIVITY OF MAMMALS

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Research Objectives: In mice exposed to ionizing radiation there is an increased incidence of tumours; however, at high doses the incidence decreases, often to a value below that observed in unirradiated mice. The object of the investigation is to test a model which explains this phenomenon in terms of the probability of radiation changing a normal cell into a potential tumour cell, combined with the probability of the radiation deleting this property. This model may lead to a better understanding of carcinogenesis and the radiotherapy of cancer.

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Progress Report: The shape of the curve relating tumour incidence to dose depends on the type of tumour and the age at which the mice were exposed to radiation. Three types of tumour are being investigated: leukaemia, which may arise from the proliferating bone marrow; lung tumours which arise from a slowly proliferating cell population; and ovarian tumours which depend on cells which cannot proliferate. Mice are given single whole-body doses of radiation at different ages, and the incidence of these tumours occurring in them are studied.

a. Leukaemia. A preliminary investigation has shown that the dose at which the leukaemia incidence is highest, is larger the younger the age at exposure. This would suggest that some of the changes in the bone marrow cells, which render it leukaemic, may result from the accumulation with age of "natural" faults, thus requiring fewer radiation events in older mice.

As a first stage in the study of the age factor, the likelihood of bone marrow cells being killed by radiation was measured by the ability of the survivors to form colonies in the spleen; two methods were used and these gave results consistent with each other. The mice were exposed to a range of radiation doses at ages 1 day, 6 weeks, 30 weeks and 2 years, and from the survival curves the D_o value, that is the dose required to reduce the cell population by a certain percentage, was determined for each age group. These values are given in table 1. This table also shows results from our earlier work, in which the dose which would kill 50% of a mouse population was determined as a function of age. There is a remarkable correlation between these figures. The apparent high resistance to radiation of 1 day old mice, is probably due to these mice being hypoxic; indeed, exposure of such mice while breathing oxygen reduces the D_o value to only 82 rads, and the LD/50 to 788 rads.

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TABLE 1

Age at Exposure	Bone marrow D_o (Rads)	LD_{50}/30 days (Rad)	Extrapolation Number
1 day	141	1068	2.5 [x]
6 weeks	94	725	2.4
30 weeks	126	959	1.4
104 weeks	119	589	0.9

[x] Measured while the mice breathed oxygen.

Another quantity determined in this experiment was the extrapolation number, which is related to the number of events required to kill the cell. As seen from the last column of the table, the extrapolation number decreases with age, thus supporting the hypothesis made above.

Once the sensitivity of bone marrow cells to radiation has been determined, it is possible to irradiate different numbers of such cells so as to ensure that the same number will survive. If these survivors' cells are then injected into mice which had been previously exposed to a sufficiently high dose, so that their own bone marrow was destroyed, the donor cells would repopulate the marrow. Any leukaemia arising in these mice later in life will be from a known number of cells which have survived different doses of radiation. This can additionally be checked by using a characteristic chromosome marker in the donor cells which is not present in the recipient mice.

In this experiment the effect due to leukaemia induction is thus separated from the effect of cell killing. By using strains of mice with a high or low natural leukaemia incidence, the relation between the induction of leukaemia and the natural

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incidence will also be clarified. This method should make it possible to achieve this in a more economical way than in large scale epidemiological studies of mice exposed to low doses of radiation.

b. Lung tumours. Preliminary experiments have shown that the incidence of lung tumours does not vary considerably with age at exposure; this may be explained by the hypothesis, supported by indirect histological evidence, that the relevant lung cell population changes very little during the life-time of the mouse. In order to test this, a technique has been developed, using an electronic cell counter with cell size analysis, to determine the number and sizes of different lung cells as a function of age and of dose of radiation.

Utilizing the fact that urethane causes proliferation of lung cells, studies are also carried out on the number and size distribution of cells in mice exposed to single doses of radiation, preceded or followed by the administration of single or fractionated doses of urethane. These data are being correlated with the incidence of lung tumours observed in mice subjected to such combined treatment with radiation and urethane.

Histochemical and enzymal characterization of the particles counted will enable us to recognize the type of cell population from which a given type of lung tumour arises. This may help in the correlation of lung tumours in mice with bronchial carcinoma in man.

c. Ovarian tumours. It has been shown that the incidence of ovarian tumours in mice exposed to radiation decreases rapidly with increasing age at exposure. This appears to be correlated with the age changes taking place in the oocyte population, which is characterized by the absence of proliferation. A detailed analysis has been carried out of the variation with age of the different types of oocyte cells, from the primary follicles to the mature cells ready for ovulation. The changes in the number of cells, following exposure to radiation, are also being studied. Once again a correlation is being sought between these changes and the incidence of ovarian tumors.

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d. Techniques. For the experiments described above several new techniques, as well as adaptations of existing techniques, had to be developed. These include the following: (i) the construction of a machine to expose automatically batches of mice to radiation, with remote control of the gas breathed by the animals, (ii) the adaptation of the Coulter counter for lung cell analysis, (iii) the development of an oxygen cathode probe technique to measure oxygen concentrations in tissue.

Significance of Research: The understanding of the mechanism of the induction of tumours by radiation, which may result from this work, will make it possible to predict the probability of a given type of tumour being produced as a result of exposure to a given dose of radiation at a given age. It would also lead to a more reliable extrapolation of results from one species to another.

This could have considerable practical applications in the assessing of the risk of exposure to small doses of radiation, and in the establishing on a firmer basis the maximum permissible doses, which at the present time are based on rather pessimistic assumptions, in the absence of reliable data. Similarly, it should make possible the more effective utilization of radiation in the treatment of cancer.

Publications:

Hawkes, M. J., Hill, R. P., Lindop, P. J., Ellis, R. E. and Rotblat, J. "The Response of C_3H Mammary Tumours to Irradiation in Single and Fractionated Doses." British Journal of Radiology, Volume 41, p. 134-141, 1968.

Town, C. D. "Effect of High Dose Rates on Survival of Mammalian Cells." Nature, Vol. 215, No. 5103, August 19, 1967.

Proukakis, C. and Lindop, P. J. "Age Dependence of Radiation Sensitivity of Haemopoietic Cells in the Mouse." Nature, Vol. 215, 655-656, August 5, 1967.

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RH 00272 (Continued)	Berry, R. J., Schwarz, G., Ellis, R. E., Lindop, P. J., Fowler, J. F. and Hegazy, M. "The Effect of Hypoxia on the Skin Response of Mice to Divided Doses of 15 MeV Electrons." International Journal of Radiation Biology, Vol. 12, No. 3, 1967.	
RH 00294-04	RADIOMIMETIC EFFECTS OF DRUGS AND INSECTICIDES	KARL SAX, SC.D. DEPT. OF BIOLOGY UNIVERSITY CENTER IN GEORGIA, INC. ATHENS, GEORGIA

Research Objectives: Chemicals known to produce mutations and birth defects may be radiomimetic and break both plant and animal chromosomes. Many drugs are suspected of inducing birth defects in humans. More than 30 such drugs are listed in Clin-Alert (August 1967). Some food additives are also suspected to be teratogenic and many insecticides are known to be radiomimetic. The simple onion root tip technique permits a rapid test of possible radiomimetic effects of agents which are water soluble.

Progress Report: Our earlier tests have shown that various plant products are radiomimetic and break onion chromosomes. These include cycasin which causes cancer in cattle who eat Cycad plants; a toxic agent in Veratrum californicum which causes birth defects in sheep if eaten by ewes during early pregnancy, and Vinblastin, a carcinostatic drug from the plant Vinca resea. We have confirmed Kihlman and Levan's early discovery that caffeine in coffee and tea is radiomimetic. We have more recently tested food additives, since a report from Japan indicates that sodium cyclamate (in Sucaryl) produces birth defects in mice. Our tests show that Sucaryl is radiomimetic at relatively high concentrations (2 cc/cup). The antioxidants, BHT and BHA, widely used to preserve the crispness of potato chips and cereal products, are radiomimetic atlow concentrations (10 ppm). Grant and Wu in Canada have found that many field insecticides are radiomimetic. We have tested two home insecticides, "Vapona" and "Bugmaster", which kill insects by filling the room with toxic vapor. They too are radiomimetic at low concentrations. We have found that

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several radiomimetic agents - coffee, alcohol and Sucaryl - are synergistic in their effect, and when combined produce about twice as many chromosome aberrations as if the effect were cumulative.

Significance of Research: Although the effect of any one of these radiomimetic agents in amounts commonly used by man, may not be hazardous, the cumulative and synergistic effect of caffeine, drugs, insecticides and food additives could increase the incidence of deleterious mutations and birth defects. There are dangers in both the "Atomic" and the "Chemical" age.

Publications:
Sax, K. and Sax, H. J. "Possible Mutagenic Hazards of Some Food Additives, Beverages and Insecticides". Japanese Journal of Genetics, April 1968.

RH 00304-05

CELL DIVISION CYCLE AND RADIOSENSITIVITY. METABOLIC STUDIES.

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Research Objectives: The main objective of the research plan is to correlate cell sensitivity to radiation damage to the metabolic events occurring during the mitotic and the meiotic cycle, with the aim of understanding the cellular mechanisms which control differential sensitivity and cellular repair from radiation damage. The study involves a biochemical analysis of the pattern of macromolecular synthesis during mitosis and meiosis in the mouse and the quantitative evaluation of some radiation effects on cells such as chromosome breakage, cell lethality and mitotic delay.

Progress Report: The research carried out so far include the study of radiation effects on germ cells and on chromosomes of somatic cells in culture. This work has been accompanied by investigations on chromosome replication, on the metabolic

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regulation of the mitotic and the meiotic cycle, and on the control of early embryonic differentiation in the mouse. It is believed that the knowledge of the basic mechanisms underlying the processes of cell growth, cell division and differentiation is a necessary condition for the understanding of the physiological and biochemical factors involved in radiation damage and repair.

1. Radiation effects on germ cells. Radiation studies on mammalian spermatogenesis are obviously very important for the implications in the genetic hazard by radiation. Among the various cell stages of spermatogenesis, the most important, from the point of view of genetic hazard by radiation, is the spermatogonial population since this population includes the stem cells, i.e. the progenitor cells of the spermatogenic line culminating in the production of the spermatozoon. The radiation response of spermatogonia in the mouse and other mammals has been extensively studied by us and other investigators. We have shown that the sensitivity of spermatogonia to radiation induced lethality varies greatly during the cycle of cell division, the stage of DNA synthesis of the cycle being the most sensitive one. In a parallel study, we have shown that the duration of the stage for DNA synthesis increases from 7 to 14.5 hours from the first to the sixth generation of spermatogonia and have suggested that this might be the mechanism of the differential sensitivity of the six generations of spermatogonia.

The existence of a stage sensitivity to radiation induced cell killing has been also demonstrated by many workers in somatic cells, but the pattern of changing sensitivity during the cell cycle has been reported to vary greatly in the various cell types. This suggests that some metabolic processes in the cell at time of irradiation rather than the state of duplication of DNA and chromosomes *per se* are important in determining the response of a cell to radiation. Among the various metabolic processes, RNA and protein synthesis might have a bearing in cell sensitivity and repair mechanisms from radiation damage. The research was therefore also oriented along these lines.

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2. Biochemistry of spermatogenesis. The most significant findings along this line were the following:

a. A decrease of the rate of RNA synthesis from the spermatogonial stem cell to the mature spermatogonium and primary spermatocyte.

b. A depression of RNA synthesis in early meiotic prophase followed by an intense activation of the rate of synthesis during middle and late prophase.

c. The absence of RNA synthesis in the condensed sex chromosomes throughout meiosis.

d. The replacement during late spermiogenesis of the somatic type histone with a very arginine-rich histone which is preserved in the nucleus until fertilization.

3. Biochemistry of the cell cycle and of early embryogenesis. Work is under progress with synchronized mammalian cell cultures to determine biochemically the pattern and time of synthesis of the various fractions of ribonucleic acid (ribosomal RNA, transfer RNA, messenger RNA) and of proteins during the stages of the cell cycle. Another line of research concerns the pattern of macromolecular synthesis during early embryonic development in the mouse. The preliminary results have shown that the synthesis of RNA which is very scarce during very early embryogenesis is rapidly switched on at the 16-cell stage until the blastocyst stage; the rate of protein synthesis remains rather low until the 8-cell stage and increases sharply afterwards in advance of the time of the activation of RNA synthesis. This research has been accompanied by electron microscopic observations on the evolution during cleavage and blastulation of the submicroscopic structures involved in RNA and protein synthesis (nucleolus, ribosomes, endoplasmic reticulum).

4. Chromosome breakage in different parts of the mitotic cycle. By using H^3-thimidine labeling to detect the position of the cell along the mitotic cycle and

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X-irradiation to produce chromosome aberrations in a mammalian cell line cultured in vitro, the following results were obtained:

a. The sensitivity of chromosomes to radiation breakage increases from G_1 (period before DNA synthesis of the cell cycle) through S (period of DNA synthesis) to G_2 (period after DNA synthesis).

b. The transition from the chromosome type aberrations to the chromatid type aberrations occurs in late G_1 before DNA synthesis; this result has been interpreted to indicate that the chromosome becomes double (i.e. splits into two chromatids) just before DNA synthesis.

c. Cells irradiated in G_2 when they reach the second metaphase after irradiation still exhibit many chromatid exchanges. This unexpected finding stimulates new ideas and interpretations of the mechanisms involved in the production of chromosomal aberrations by radiation.

Significance of Research: The increase of the present scientific knowledge on the mechanisms which control cell sensitivity to radiation damage and cell repair is the necessary premise for the control of radiation hazard in man. In particular, the part of the research plan concerning the study of radiation effects on germ cells is very important for the implications in genetic hazard by radiation. The radiological therapy of tumours might also greatly benefit of a better knowledge of the mechanisms underlying cell sensitivity to radiation in different parts of the life cycle of the cells.

Publications: No additional publications.

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(2) RH 00325-02	DIFFERENTIAL RESISTANCE OF CELLS TO IRRADIATION	CARL C. SPEIDEL, PH.D. DEPT. OF ANATOMY UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VIRGINIA

Research Objectives: Extensive studies of the effects of radiation on cells were undertaken starting in 1953. The injurious effects of X-rays and of ultraviolet rays were first observed on frog tadpoles. Next the injurious effects of these rays were observed on a one-celled animal Tetrahymena which was discovered to be a parasite of weakened frog tadpoles, a "facultative parasite". The third animal selected for experiment and study was the sea urchin Arbacia, special attention being given to the early developmental stages from the fertilized egg to the free-swimming larva. Again both X-ray and ultraviolet radiation were used. Finally, the effects of four radiomimetic chemical compounds on developing sea urchins were also studied. The four compounds were caffeine, colchicine, nicotine, and trichloroacetic acid. Results obtained with these compounds were compared with those caused by radiation.

Thus, radiation effects have been studied on (1) vertebrate cells, as represented by frog tadpoles, (2) single cells which are also entire animals, as represented by the protozoan Tetrahymena, and (3) gametes (eggs and sperms) and ensuing stages of early development, as represented by sea urchins. These investigations have afforded a broad basis for an understanding of some fundamental changes in structure and behavior of cells exposed to radiation.

Satisfactory permanent records of the principal histological changes were obtained by cine-photomicrography. Time-lapse motion pictures taken at suitable speeds using high magnification were particularly valuable for recording slow cellular movements.

Progress Report: The results of these studies have been published in a series of 30 carefully worded abstracts. Of these, eight deal with tadpoles, six with Tetrahymena, and the rest with sea urchins exposed either to radiation or to radiomimetic chemical

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compounds. All of the investigations on sea urchins were made in collaboration with Dr. Ralph Holt Cheney, an expert in handling these animals. One study of mating tetrahymenae was made with Dr. George Gilbert Holz, Jr..

The following comments indicate briefly the rather wide range and significance of some of the results reported in abstracts (as mentioned above). The subject areas covered by these abstracts are:

1. The X-ray dosage range and the tissues injured in tadpoles.

2. Representative motion picture scenes selected for exhibition.

3. The effects of X-ray treatments on Tetrahymena corlissi, particularly as these affect asexual (binary fission) reproduction (controlled by the macronucleus) and sexual reproduction (controlled by the micronucleus). Binary fission continues even though the micronucleus has been eliminated by the radiation. Some animals survive even after single doses of up to 600,000 roentgens. Their resistance to radiation (about 1,000 times that of man) is probably due to their polyploid macronucleus, a structure which contains several sets of genes. They may also survive after many repeated single doses given at intervals of about two weeks, totaling 13 million roentgens, or even more.

4. Mating Types I and II of Tetrahymena pyriformis will still mate even after each type has been subjected to radiation totaling 24,000 kiloroentgens, given in four weekly doses of 6,000 kiloroentgens each.

5. Specific damaging effects induced in developing sea urchins derived from eggs and sperms subjected to ultraviolet or to X-rays.

6. Effects of ultraviolet radiation on eggs under static and non-static conditions and on sperm. The differences in results are explainable on the basis of size and motility.

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7. Results of ultraviolet treatment of regenerating tail in tadpoles in bringing about supernumerary notochords, deflected spinal cords, and extra fins.

8. Variations of the micronucleus induced in Tetrahymena corlissi by ultraviolet treatments. These are of interest for comparison with X-ray effects. The micronucleus is the chief center for DNA (deoxyribonucleic acid), which is now generally regarded as most important in the transmission of hereditary characteristics from generation to generation.

9. An account of an important series of experiments on sea urchins bearing upon the differential resistance of young zygotes to irradiation. Five specific time stages between fertilization and first cleavage of the egg are featured. The observed differential resistance at these stages is linked with the state of deoxyribonucleic acid (DNA) in the cell at each time stage.

10. Abnormalities induced in sea urchin progeny by four radiomimetic chemical compounds. Of special interest were colchicine, which profoundly affects cell division (mitosis), nicotine, which alters the surface of the sea urchin egg, and trichloroacetic acid, which inhibits the formation of proteins. Radiomimetic-induced abnormalities were compared with those induced by X-rays.

The damaging effect of nicotine on the sea urchin egg surface was followed by distorted cleavage. This led us to think that nicotine might, over a long period of time, damage cells that line the respiratory tract in man and thus be a contributory factor in causing lung cancer in habitual heavy cigarette smokers. Entrance of viruses into cells would be expedited, which by altering the DNA could lead to uncontrolled cell division and multiplication.

A major stated aim was to record by cine-photomicrography the principal results obtained. This has been achieved. A selection of the best scenes resulted in the production of 23 reels, each one 400 feet in length, 16 mm silent, running time 16 minutes. Each reel is fully edited with descriptive titles. Of these, five deal

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with frog tadpoles, three with Tetrahymena, and 15 with sea urchins. Nineteen exhibitions of these motion pictures have already been made mostly at the Woods Hole, Massachusetts, Marine Biological Laboratory, and at Annual Meetings of the American Association of Anatomists, and of the American Society of Zoologists. These exhibitions have been well received and have stimulated discussion and interest.

Significance of Research: In conclusion, several items may be stressed. The motion picture reels constitute a unique and valuable group of records which demonstrate the visible effects of two kinds of radiation on the cells of representatives of three different phyla of the animal kingdom - vertebrate tadpoles, protozoan tetrahymenae, and echinoderm sea urchins.

Tadpoles and tetrahymenae. The discovery that a new species, now classified Tetrahymena corlissi, could invade weakened tadpoles made it possible to subject experimentally-infected tadpoles to X-ray irradiation. Suitable doses that seriously injured the tadpole host, injured the tetrahymena parasites only slightly or not at all. This favored more rapid destruction of the host tissues.

Deep radiation is often resorted to in cases of human cancer, as for example in radiating cancerous internal viscera with the moving cobalt source technique. It has occurred to me that such treatment is likely to be more injurious to both cancerous and normal cells, than to any pathogenic protozoa, or bacteria, or viruses, that might be present nearby. In individuals harboring such organisms or viruses, death might be hastened, just as it is in the tadpoles infected with tetrahymenae.

Sea urchins. Our investigations have emphasized the role of the irradiated sperm in causing the observed abnormalities that develop in the offspring. Many investigators have limited their experiments almost completely to those on eggs and early zygotes. Our emphasis on the role of radiation-damaged sperm has enabled us to analyze and clarify the extent to which the nuclear constituents (chromosomes and DNA) and the cytoplasmic constituents are responsible for the abnormalities that later appear in the progeny.

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A feature of our work is that after fertilization all progeny are kept under observation up to the free-swimming larval stage (2-4 days). This is important as it allows us to make better analyses than are possible with observations limited to one day or less. Such prolonged observations have led to our discovery of varieties of embryonic autotomy (self separation of a part). Significant differences in the types and locations of cells to be cast off by autotomy were noted and correlated with X-rayed and ultraviolet-rayed gametes respectively.

Publications:

Speidel, C. C. and Cheney, R. H. "Nicotine Effects on the Gametes of Arbacia Punctulata." The Biological Bulletin, Vol. 133, No. 2, p. 486, October 1967.

Speidel, C. C. and Cheney, R. H. "Trichloroacetic Acid Effects on Gametes and Zygotes of Arbacia Punctulata." American Zoologist, Vol. 6, No. 4, November 1966.

(1) RH 00328-03

ANCESTRAL VS DIRECT EFFECTS OF IRRADIATION ON MAMMALS

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DEPT. OF RADIOLOGY AND RADIATION BIOLOGY
FORT COLLINS, COLORADO

Research Objectives: The long term study on the effects of nearly continuous exposure (20-22 hours per day) to low dosage levels of gamma radiation from Cobalt-60 on the general well being and duration of life of a population of mice has been continued.

Progress Report: A population of ten inbred strains, four single pair matings per strain, were originally exposed to an average dosage level of irradiation of 0.12r per hour. Five strains failed to maintain themselves beyond five generations. Two of the remaining five maintained themselves seven years, 13 and 16 generations, one for nine years or 31 generations, one appears unable to carry on beyond generation 28 and one is still very vigorous after 38 generations of nearly continuous

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irradiation for 10 years. All data covering complete histories of reproduction and life duration for each successive generation have now been put on punched cards in a format compatible for use with the generalized data storage and retrieval system of the Biometry Section of the Collaborative Radiological Health Laboratory. Utilization of this system will permit easy access to the data in the course of performing additional analyses.

Significance of Research: Generations have been continued by within litter matings. Only two visible genetic changes have been observed. Brachypodia was found in progeny of the 10th generation in the BaB strain. A more extreme abnormality appeared in the progeny of a 23rd generation brother x sister mating that had been removed from the radiation facility for testing. This pair had 40 progeny, eight of them were abnormal and dead at birth. The abnormality was recognized by shortened extremities, the legs and feet appeared paddle-like. A stock for this condition has been maintained. It is inherited as a simple recessive and is lethal at birth or shortly after when homozygous. Cleared specimens of the paddle-like condition indicate abnormal skeletal development. Bones are shortened, ribs are missing, fused together and branched to varying degrees. Bones in the legs and feet also appear fused together or missing. Dr. Donald J. Nash (Zoology Department) has observed cleft palates in those he has studied.

Publications:

Gowen, J. W. and Stadler, J. "Life in Environs of Continuing Higher Levels of Added Radiant Energy from Puberty to Death as Expressed by Mice." Environmental Research, Vol. 1, No. 2, October 1967.

Gowen, J. W. and Stadler, J. "Genetic Characteristics Influencing Vaccine-Conferred Immunity." Journal of Infectious Diseases, Vol. 117, 129-150, April 1967.

Gowen, J. W. and Stadler, J. "Specificity of Vaccine-Conferred Resistance to Salmonella Typhimurium in Mice." Journal of Infectious Diseases, Vol. 117, 151-161, April 1967.

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RH 00330-04	LOW LEVEL GAMMA RADIATION AND PHYSIOLOGICAL PERFORMANCE	GEORGE M. KRISE, PH.D. RADIATION BIOLOGY LABORATORY TEXAS A&M RESEARCH FOUNDATION COLLEGE STATION, TEXAS

Research Objectives: These experiments are designed to test the effects of virtually continuous (20 hour per day) exposure to various relatively low doses of ionizing radiation on the ability of the test animal, the Spanish Goat, to perform both reproductively and physiologically. The doses of ionizing radiation in use are 0, 2, 7, 15, 30 and 40 R per 20 hour day. The measurements taken to weigh the effects of these exposures on performance are: survival; changes in body weight and growth; reproductive performance in the female as measured by estrus, pregnancy, and kids produced; reproductive performance in the male, measured by semen analysis and ability to induce conception in females; changes in electrocardiogram; respiration and other physiological parameters such as EEG and analysis of peripheral blood.

Progress Report: Because of the conflicting reports in the radiation literature concerning the comparative radiosensitivity of the goat, we first chose to establish the dose that could kill fifty percent of a test population (LD_{50-30}) in thirty days. Male goats were irradiated at doses of 300, 400 and 500 R (at 85R/hour). The calculated lethal dose fifty percent (LD_{50}) for these animals was approximately 550R. This dose compares favorable with the estimated LD_{50} dose for man and with some of the published results for the goat. Since some animals survived the LD_{50-30} study, it was decided to test the premise that a conditioning dose may alter the later radiosensitivity of test animals. Sheep, swine, and laboratory animals (rats, mice) had been tested in this manner at the Naval Radiological Defense Laboratory and they were found to exhibit an increased radioresistance to acute exposure up to at least 100 days post-conditioning dose. The survivors of the LD_{50} studies were exposed along with a control group of goats to 40R per day until death. Even though we are working with a rather heterogenous group of animals, there appeared to be neither a decrease nor an increase in radioresistance due to the conditioning dose. These results might

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be interpreted in several ways. However, recent evidence would seem to point to the fact that the dose rate at which the conditioning dose is given is critical. Since our conditioning dose was delivered at a relatively low rate, it might be concluded that repair mechanisms were in operation throughout the period between conditioning and test dose to such an extent that very little latent damage or hypertrophy remained.

Survival times of both male and female goats exposed to 40, 30, 7 and 2 R per 20 hour day have been calculated. The time required for half of the animals to die when exposed to 40 R per day was 56 days for males and 50 days for females; 30 R - 101 days for males and 86 days for females; 7 R - 384 days for females (half of the males have not died to date). To this date, neither half of the females nor males have died in the group exposed to 2 R per day.

Fertility tests of the animals exposed to 2 and 5 R per day indicates that after two years of exposure to these dose rates that both female and male goats remain fertile. All females of both exposure groups have produced kids each breeding season.

The males in both groups have suffered declines in the number of sperm but have not as yet reached clinical sterility.

The kid crop from females exposed to 5 and 2 R per day appeared normal and the kids produced by these mothers appear normal except that their growth rate, as measured by weight gain, appeared to be somewhat depressed. Fertility of the kids produced from parents irradiated at 2 and 5 R per day have not been tested. The effects of pre- and post-natal irradiation received by these offspring might be expected to affect their life expectancy, however, this endpoint will not be evaluated.

An opportunity to check for changes produced in the chromosomes of the 2 and 5 R irradiated animals arose but the results were virtually negative. Either repair of chromosome damage occurs rapidly or no significant damage results from these dose rates.

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Significance of Research: Should eventualities result in widespread contamination of the world or limited parts of it by radioactive materials, the environmental background could be raised for significant periods to levels approaching those used in our experiments, i.e., 40, 30, 7 and 2 R per day. It is highly desirable to know how man and his domestic cohorts would react to these changes in the environment. The experiments will give information from which judgements could be made concerning the reaction of both man, his domesticated food animals and wild game to such changes in environmental radiation. Such information would help in future planning concerning projected shelters, protection and treatment of both man and the animals on which he is dependent for animal protein.

Publications:

Szabuniewicz, M., Davis, R. H., Jr., and Brown, S. O. "The Terminal Electrocardiogram In Irradiated Goats." The Southwestern Veterinarian, Vol. XXI, No. 2, 1968.

Austin, J. W., Hupp, E. W., Krise, G. M. and Brown, S. O. "Semen Production of Continuously Irradiated Spanish Goats." Radiation Research, Vol. 31, No. 3, July 1967.

Krise, G. M., Hupp, E. W. and Austin, J. W. "Survival of Goats During Continuous or Interrupted Continuous Irradiation." Abstract Ab-1, Radiation Research, Vol. 31, No. 3, July 1967.

Szabuniewicz, M. and Clark, D. R. "Analysis of the Electrocardiograms of 100 Normal Goats." Am. J. Vet. Res., Vol. 28, No. 123, pp. 511-516, March 1967.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00345-04	PROTEIN METABOLISM IN NORMAL AND IRRADIATED LENSES	MORRIS GREEN, PH.D. DEPT. OF BIOLOGICAL SCIENCES HUNTER COLLEGE NEW YORK, NEW YORK

Research Objectives: Our present work is concerned with amino acid permeability and protein metabolism in normal as well as in cataractous lenses induced by X-irradiation or galactose feeding. Both the permeability and the metabolism are altered during cataractogenesis. It is our intent to determine whether these changes are primary or secondary effects of the treatments, and elucidate the specific causes leading to these alterations. The biochemical parameters under investigation include: a) amino acid transport and metabolism, b) enzyme systems involved in carbohydrate, amino acide, and protein metabolism, c) nucleic acid synthesis.

Progress Report: Our studies on amino acid permeability of the lens show that both X-irradiation and galactose feeding lead to decreased accumulation of amino acids prior to the occurrence of lens opacity. This alteration is associated with an increase in leakage of amino acids from the lens and possibly a failure in energy metabolism necessary for the net accumulation. The carrier system(s) for the amino acids studied appear to be unaffected in the early stages. Whereas a variety of metabolic inhibitors studied also decrease amino acid accumulation, they do not appear to increase leakage in the lens.

Amino acid incorporation into protein increases in the early stages of opacification concommitant with an increased activity of an enzyme, methionyl-RNA-synthetase, and of thymidine incorporation into DNA. Further studies indicate that the energy supply for amino acid transport and protein metabolism involve two discrete energy pools.

Since X-ray damage has been equated with accelerated aging, all of our parameters have been investigated as a function of age. Amino acid transport and its incorp-

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oration into protein as well as NAD-dependent -glycerophosphate dehydrogenase concentration decrease with animal age. On the other hand, methionyl-RNA-synthetase activity remains constant. Amino acid leakage from the lens increases with aging.

The current use of an amino acid analyzer enables us to quantitate the free amino acids in normal, precataractous, and aging lenses, and to determine which series of amino acid transport carriers are affected or not affected under pathological conditions. Not all amino acids are found to be affected to the same extent by aging and cataractogenesis. Glutamic-oxaloacetic acid transaminase (GOT), an enzyme well known to be involved in both amino acid and carbohydrate metabolism is currently being investigated for its possible role in cataractogenesis.

A new method developed to study efflux of amino acids from lenses allows transfer of lenses through a series of media with minimal trauma and yields highly reproducible data. This method eliminates many of the inherent errors involved in efflux determinations in lens research; it also provides a method of simultaneous evaluation of all components (diffusion, exchange diffusion, and active transport) involved in amino acid transport. Alterations in lens permeability can now be studied with respect to each of these transport mechanisms.

Significance of Research: Exposure to ionizing radiation results in the formation of lenticular opacities which are associated with impairment of membrane transport and lenticular metabolism. Although certain biochemical changes that occur during cataractogenesis are similar to those encountered in aging and metabolic diseases, others are not. By elucidating various parameters of cell maintenance (transport, nucleic acid synthesis, enzyme systems) and determining the time sequence of alterations, one ought to be able to determine the primary and secondary causes(s) leading to the development of opacity after exposure to ionizing radiation.

Publications: None

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RH 00364-02	EEG AND BEHAVIORAL EFFECTS OF GAMMA RADIATIONS	DANIEL E. SHEER, PH.D. DEPT. OF PSYCHOLOGY UNIVERSITY OF HOUSTON HOUSTON, TEXAS

Research Objectives: A primary objective of the research is to develop a clearly defined index of electrical activity which can be followed at different levels of the brain -- peripheral, subcortical, and cortical -- to investigate effects of irradiation on the nervous system. Other objectives are concerned with establishing relationships between such an index, expressed in quantitative terms, and concomittant behavioral effects of irradiation.

Progress Report: Procedures have been developed for multi-lead electrode implantation in the brain, behavioral testing and analysis of the electrical activity recorded from different brain sites. The analysis of the electrical activity is performed on an analog computer system developed in our laboratory. The output from the computer provides multi-channel continuously moving power spectre from one brain site or cross-power spectre from two brain sites. Changes in the electrical activity can be continuously related to changes in behavior before and after the effects of irradiation. Committant electrical activity and behavior is being investigated, at head only and whole body graded irradiation doses.

Significance of Research: The establishment of a specific, reliable index of electrical activity, which can be expressed quantitatively and followed at different levels of the brain would offer considerable promise both as a diagnostic tool and for making connections with other effects of irradiation, such as metabolic, vacular, and histochemical analyses.

Publications: None

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(1) RH 00390-03	EFFECTS OF AGING AND RADIATION ON HAARSCHEIBE PIGMENT	P. DONALD FORBES, PH.D. DEPT. OF BIOLOGY BARRINGTON COLLEGE BARRINGTON, RHODE ISLAND

Descriptor: The effect of radiation on skin pigmentation is being studied in mice.

Publications: None

RH 00391-03	AGING IN X-RAY IRRADIATION AND NON-IRRADIATION	ROGER T. DAVIS, PH.D. DEPT. OF PSYCHOLOGY UNIVERSITY OF SOUTH DAKOTA VERMILLION, SOUTH DAKOTA

Research Objectives: The purpose of this research was to better understand how we age and to separate factors of aging that were inevitable from those that resulted from severe radiation and from the effects of our own attitudes about being old. This was done by carefully observing aging in a group of monkeys and in their offspring and by periodically comparing older and younger animals on tests derived from what we can understand about aging in human beings. Since the monkeys were not effected by attitudes toward aging their performance should give us a good estimate of unavoidable aging.

Progress Report: The studies of aging at this laboratory grew out of a series of experiments, begun in 1952 which were concerned with the effects of large doses of whole-body X-ray radiation on behavior of monkeys. Subsequent experiments dealt with the chronic effects of radiation and with the similarities and differences between radiation and drug effects (see Davis, 1965, for a summary of research on the radiation syndrome in monkeys). Ultimately the hypothesis was examined that the effects of radiation and those of aging were similar, and the testing of this hypothesis was the major topic of this report.

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The experiments in this report were conducted between 1965 and 1968 using the monkeys that had survived radiation, nonirradiated controls, and the offspring of these older animals. The irradiated animals and the nonirradiated controls were approximately 15 years old in 1965, and the progeny between 7 and 9 years old. The old animals were trained nearly continuously from 1952 to the present and every animal received the same tests and, as nearly as possible, the same amount of training on each test. Beginning in 1959, the progeny were trained on experiments that paralleled these given to the old animals.

By the time the oldest animals were middle aged, we had established that: (a) radiation and aging did not have similar effects, (b) animals performing best on learning set problems (analagous to problems of thinking and reasoning in human beings) prior to radiation were more likely to die from the effects of radiation than animals that had performed poorly on these tasks, and (c) survivors of radiation and their nonirradiated controls became quite dissimilar in social and self directed behavior over a period of seven years after radiation.

After these studies we shifted the animals to a project concerned with perception. Although not designed to assay aging differences, the perception studies clearly showed that the three groups of animals differed in their perceptions of size, distance, and the discrimination of cues widely separated from responses. These studies in perception, along with those asking the question about the similarities and differences of radiation and aging, plus the longitudinal studies on aging, formed the context for the present studies.

Six general hypotheses about human aging were submitted to monkey tests: (a) do old animals have more difficulty shifting set than young, (b) does advancing age interfere with memory, (c) are there systematic perceptual changes with advancing age, (d) does the ability to track moving targets decline with age, (e) does work output decrease as animals get older, and (f) does the general social, self-care and exploratory behavior of the animal change with passing years?

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a. Shift of set. One of the hypotheses examined earlier at this laboratory was that old animals, especially those surviving radiation, shift set less well than young animals (Davis, 1963, Experiment-3). This was confirmed tentatively, so two more studies were conducted recently to examine the nature of this difference. One study used discrimination reversal problems and the other required young and old monkeys to shift from one mode of response to another.

There is a reasonable amount of evidence that supports the idea that discrimination reversal problems are sensitive to species differences and distinguish between normal and brain damaged animals. The present experiment extended the use of reversal problems to a comparison of old irradiated, old nonirradiated and young monkeys. The 144 reversal problems given to each animal contained either 3, 4, 5, 6 or 7 preshift trials followed by 4 postshift trials. On the preshift trials of any problem, the monkey was given a unique pair of common objects (e.g., oil can and rubber heel) to discriminate. The experimenter arbitrarily designated one object in each problem as correct and the other as incorrect. He rewarded the animal for selecting the correct object, but withheld reward if the animal chose the wrong object. At the beginning of the postshift trials, the correct and incorrect designations were reversed, and the previously incorrect object followed the position of the food. Since the animals did not know whether the shift was coming on the 4th, 5th, 6th, 7th, or 8th trial, they usually made an error on the first postshift trial and until they had had quite a few reversal problems, continued to make errors on the other postshift trials.

The young animals made more preshift errors than the old animals, but the old irradiated animals made more postshift errors than the nonirradiated animals (both old and young). This effect was clearly related to improvement of postshift performance of the nonirradiated monkeys with practice and failure of the old irradiated animals to learn how to shift.

Tests of flexibility or rigidity required human beings to shift from one strategy of problem solving to another, e.g. the Luchin's Jar Test, the Wisconsin Card Sorting

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Test, the Vigotsky Test, etc. The flexibility-rigidity dimension was examined in monkeys by requiring them to shift between two different response sets and at the same time shift in the type of cues used to respond. One response set was to use signs to go to particular positions, i.e. on one trial a pair of identical stimulus objects was a sign to choose the one on the left while on another trial an alternative pair meant go-Right. The other set was to approach one of two dissimilar objects. The two cue types were colors and forms. These conditions were presented in the following way with the second reversal being oritical.

	Original training	First reversal	Second reversal
Sets	Sign	Approach object	Sign
Cue type	Color	Color	Form

The three groups of animals did not differ reliably in either the original training or in the first reversal, but all of the young animals met the criterion of training in the second reversal faster than any of the old animals.

Simple shifts of set were thus specific to chronic radiation effects whereas complex shifts involving mode of response and set differentiated young and old. The former is probably the case of healthy individuals vs. those less well while the latter may be an unavoidable change in intellectual functioning.

b. Age and Memory. Loss of memory is the most familiar symptom of senile change, but memory loss has been studied only to a limited extent with human beings and very little with animals. Twenty different experiments were conducted on memory with the present groups of animals. Most of them were variants of the widely used direct delayed response problem which may have involved attention more than memory. Another experiment was analagous to a verbal memory experiment (paired associates) commonly used with human beings, while three experiments involved memory for form.

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The latter studies are still in progress and analysis of the existing data has added new dimension to our studies since the last official progress report (January 19, 1968).

Delayed response tests repeatedly have separated animals with frontal lobe brain damage from nonoperated animals. This test simply involves showing an animal which of two food recepticals in a formboard contains food, covering the recepticals with a pair of identical objects, and waiting for a prescribed period of time, and allowing the animal to make a choice. Since the stimulus objects are identical to each other it is necessary for the animal to attend to the baiting and to remember where the food was placed.

None of the direct delayed response tests differentiated young and old animals or were sensitive to radiation effects. Delays ranged from 0.5 to 90.0 sec. and a number of procedural variable were studied. Factor analysis indicated that the variants grouped into several independent tests.

Monkeys were trained on concurrent discriminations to discriminate pairs of pictures cut from magazines. The basic procedure was analagous to memory experiments using pairs of nonsense syllables, but instead of syllables there were two pictures, one of which was rewarded the other not rewarded. As in experiments on human memory a number of paired stimuli were given successively and the animal had to repeat all pairs of stimuli to discriminate a particular pair again. This continued until an animal associated each of eight pairs of pictures errorlessly on two repetitions of a "list". Then a retest was given after 0, 1, 24 or 168 hours and scores of per cent savings were computed (number of repetitions on first learning - number of repetitions on second learning/number of repetitions on first learning). Memory for these patterns was very good at intervals up to a week, but there were no reliable differences in the long term memory of young or old animals and the prior condition of radiation did not significantly affect performance.

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Two experiments have been completed on pattern memory and two more are in progress. They used a display consisting of 16 boxes arranged in a 4 x 4 grid. Squares of translucent white plastic were the doors to the boxes and each box could be illuminated separately. Monkeys were trained that a light in a box indicated food reward, and the order of lighting was controlled by computer program. A trial was as follows: the appropriate boxes were baited, the animal was allowed to view the light or combination of lights for 1 sec. through a clear screen, and the lights were turned off. After a time delay of 0.5, 1, 2, 5, 10 or 20 sec. the clear screen was raised and the animal had to remember the correct pattern of lights.

In the first experiment there was only one light on a particular trial, in the second experiment there were always two lights per trial, and in the third experiment there were four. The difficulty of this task can be appreciated by constructing 4-cell patterns, viewing each for 1 sec. and reproducing the patterns after the prescribed delays.

The results have been dramatic. There was rapid loss in memory especially during the first few seconds and the loss was much greater in old than young animals. The old irradiated animals show even greater memory loss. This is especially notable since the young and old animals do comparably well on direct delayed response problems succeeding most of the time with delays of as much as 90 sec. It appears that the difference is related to the complexity of the task.

c. Perceptual changes with age. A number of differences were found between the three groups in their use of cues. One was that old animals use colors as cues much more than young but only if the colors are highly saturated. Another finding was that when given conflicting cues the young animals were much more likely than the old animals to select one set of cues and respond to them without regard for the other set. However, both young and old animals responded comparably to illusory stimuli.

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d. Tracking. Since the tracking task did not depend on prior learning in the laboratory all of the animals in the colony were employed. They ranged in age from 2.0 to approximately 17 years of age. Old and young animals were comparably proficient on this task although the youngest and oldest animals were much more variable in response than animals of middle age.

e. Work output. This was studied to see if old animals would sustain as much work as the young animals. Surprisingly there were no reliable differences.

f. Longitudinal studies. In addition to the above experiments, we continued our longitudinal studies to a span of 14 years. Generally the differences that we had noted between irradiated and nonirradiated animals after 7 years were accentuated after 14, e.g. old animals, either irradiated or not, decreased manipulating the objects in their environment by a factor of 90 per cent. Social behavior decreased from 58.6 to 14.5 per cent of all behavior of the nonirradiated animals over a 14 year span whereas there was no reliable decline in the irradiated monkeys. Other than this difference, the two groups of old animals showed similar age trends and the trends were toward less contact with the environment.

Significance of Research: As we get older all of us find it harder to remember, are more set in our ways and have difficulty understanding new ideas. We also may get gloomy, discouraged and disillusioned. Are these changes an unavoidable part of aging or do they merely reflect our attitudes and fears about aging? Do they come from within us or are they due in part of the attitudes that society has about aging? There have been sociological studies of these problems but they have the limitation that man does not age without social and attitudinal factors being present -- they are part of civilization. Therefore to find out what changes in aging are free from attitudes we have studied aging in monkeys who are very similar to us anatomically, who have a high level of intelligence, but who don't talk. By better understanding behavioral changes

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that are, and are not, intrinsic to aging we might be able to make the lot of the aged easier through a program of eduction aimed at correcting misconceptions.

Publications: None

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RH 00400-01

PROLONGED PRIOR TRAINING AND RADIATION RESISTANCE

WILLIAM W. HEUSNER, PH.D.
DEPT. OF HEALTH, PHYSICAL EDUCATION & RECREATION
MICHIGAN STATE UNIVERSITY
EAST LANSING, MICHIGAN

Descriptor: The purpose of this investigation was to study the effect of various levels of pre-irradiation physical activity upon the radiation resistance of animals. Rats were used and received 650R of X-irradiation.

Publications: None

(1)
RH 00437-02

STUDY OF RADIATION EFFECTS ON CHROMOSOMES

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DEPT. OF RADIOLOGY
UNIVERSITY OF ROCHESTER
ROCHESTER, NEW YORK

Research Objectives: The ultimate objective of this research is to understand how radiation causes bodily injury so that such injury in mankind such as leukemia and thyroid cancer due to inevitable exposure to man-made radiation can be prevented or treated. Since all manifestations of radiation injury in man stem from radiation damage of individual cells, we have focused our attention on cultured mammalian cells, the simplest of all mammalian systems. In our research, we aim specifically to explore the functions of chromosomes (the genetic apparatus of cells) and their role in producting radiobiological damage, for example, in cell killing, mitotic delay and chromosome aberrations.

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Progress Report:

1. Synchronization of Mouse Leukemic, L5178Y, Cells with a Combined Method of Excess Thymidine and Colcemid

A mammalian cell passes through several stages in its life cycle (G_1, S, G_2 and M stages) before it divides into two G_1 stage cells. Since it is now known that the cell does not respond to ionizing radiation as well as to other damaging agents in the same way in the different stages of its life cycle, it is important to identify the various molecular events occurring in the different stages of the cell's life. One way to study the events in each of the four stages is to use a synchronized population, i.e. a population with all cells in the same stage of the life cycle. Since no one has been able to synchronize the growth of our cell line (mouse leukemic cells, L5178Y), it was necessary to develop a method of achieving synchronization of cell growth. This was accomplished by adding excess thymidine to cause cells to accumulate in S stage; following this, the cells were treated with colcemid to allow them to accumulate in the narrow region of metaphase in the M stage. The degree of synchronization achieved was as good as most of the other recent methods developed for other cell lines and the method has the advantage of achieving synchrony within one cell generation.

2. Switching-on and Switching-off Time of Nuclear RNA Synthesis

One of the significant events that occur when cells pass from G_2 to M stage is the coiling of the uncoiled chromosomes. Since reversion of the coiled chromosomes to the uncoiled state has been suggested as one of the main causes of radiation-induced mitotic delay, the transcription of m-RNA, a molecular event which might be associated with such transition of uncoiled to coiled state of chromosomes, was studied. It was observed that transcription of m-RNA (nuclear RNA synthesis) was switched off 12 ± 6 minutes before the beginning of the M stage; the nuclear RNA synthesis was switched on again at the end of mitosis. Moreover, it was concluded

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that, since the radiation did not interfere with the switching-off of nuclear RNA synthesis, the transition between uncoiled chromosomes and coiled chromosomes could not be the cause of the radiation-mitotic delay.

3. Radiation-induced Mitotic Delay Phenomena

An immediate response of the rapidly dividing cell population to exposure to radiation is the decline of the mitotic index (the fraction of M stage cells). Various events in the G_2 stage were examined in an attempt to identify molecular mechanisms associated with and possibly responsible for this phenomenon. Radiation induces a blockage of cell progress through its life cycle at the middle of G_2 stage (as previously shown by Puck and Steffen in human carcinoma HeLa S3). Actinomycin D (0.5 ug/ml) which stops RNA synthesis was found to induce a block in the life cycle at the beginning of G_2 stage, and, in higher concentrations (5 ug/ml) caused another block at the end of G_2 stage. Since the time of the actinomycin block did not coincide with the radiation block, the biochemical effect induced by actinomycin, i.e. block of RNA synthesis, seems unlikely to be the cause of the radiation block. On the other hand, treatment of the cells with puromycin which stops protein synthesis induced a block at the same time as the radiation block; it seems likely, therefore, that interference with protein synthesis is a factor in the radiation block.

In order to prove that protein synthesis is involved in the mitotic delay phenomena, cells were irradiated and effects of actinomycin D, dinitrophenol and puromycin on the life cycle were studied to see if they would interfere with the recovery of cells from the mitotic delay phenomenon. It was found that the only agent which delayed the recovery of the mitotic index was puromycin (inhibitor of protein synthesis). Recently, a somewhat similar conclusion was reached by Petersen and Walters in Chinese hamster cells (Biophysical Society Meetings, 1968). This agreed with the previous results of Rustad and his associates in studies of the radiation-induced division delay of sea urchin eggs.

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4. Effects of Actinomycin D and Puromycin on Cell Progress through Cell Cycle

By using synchronized cell populations, the effects of actinomycin D and puromycin were examined. The purpose of this experiment was to examine whether or not m-RNA synthesis and protein synthesis are required for cells to progress through the cell cycle. Cells divided normally in the presence of actinomycin D and puromycin suggesting that m-RNA synthesis and protein synthesis are not necessary for M stage cells to enter G_1 stage.

However, when the cells entered G_1 stage, they failed to take up uridine-H^3 (a precursor of RNA), but incorporated leucine-H^3 (a precursor of protein) even in the presence of actinomycin D. It was concluded, therefore, that newly divided G_1 cells are able to synthesize proteins even without synthesis of m-RNA. This suggests that the daughter cells carry over m-RNA from the previous cell cycle and that the new G_1 cells are able to utilize the m-RNA of the mother cells to synthesize new proteins.

In the presence of puromycin, the G_1 cells failed to take up either uridine-H^3 and leucine-H^3. This suggests that puromycin inhibited protein synthesis and m-RNA synthesis, and that further m-RNA synthesis required protein synthesis in the newly divided G_1 cells.

Finally, the newly divided cells in the presence of actinomycin D and puromycin failed to enter S stage. Thus, new m-RNA synthesis and new protein synthesis in the young daughter cells seem to be necessary for new DNA synthesis to be initiated.

5. Induction of Chromosome Aberrations by Excess Thymidine and Repair by Deoxycytidine

Chromosome aberrations were induced by treatment of cells with excess thymidine which could be repaired by post-irradiation treatment with deoxycytidine. The type of chromosome aberrations induced by X-rays are similar in nature to those in thymidine-treated cells but are not repaired by deoxycytidine. The molecular mechanisms of excess thymidine-induced chromosome aberrations are now under investigation.

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RH 00437 (Continued)

Significance of Research: 1. Radiation-induced mitotic delay, a well known cellular radiobiological effect, has now been shown to be caused by inhibition of the synthesis of protein(s) necessary for mitosis. (The same conclusion was reached simultaneously in Chinese hamster cells by Walters and Petersen.) 2. In many biological phenomena (aging, carcinogenesis, transformation of stem cell to differentiated cells, etc.), the regulation of the rate of cell division plays an essential role. In these situations, either the duration of G_1 stage or the G_2 stage can be directly related to rate of cell division. By mapping the time of various molecular events in cell cycle, it may be possible to identify the regulatory mechanisms of cell growth.

Publications:

Okada, S. "A Simple Graphic Method of Computing the Parameters of the Life Cycle of Cultured Mammalian Cells in the Exponential Growth Phase." The Journal of Cell Biology, Vol. 34, No. 3, pp. 915-916, 1967.

Okada, S. and Doida, Y. "Determination of Switching-off and Switching-on Time of Overall Nuclear RNA Synthesis." Nature, Vol. 216, October 21, 1967.

RH 00467-02 (Formerly RH 00068)

RADIATION EFFECTS UPON NERVOUS SYSTEM AND BEHAVIOR

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Research Objectives: Ionizing radiation produces: (a) immediate transient effects upon behavior and electrical activity of the brain, by stimulating the olfactory system and perhaps other peripheral receptors, (b) more enduring effects upon behavior which resemble the effects of noxious drugs and toxins, presumably produced by the products of energy absorption in the fluids and tissues of the body. Our research plan is to elucidate the relevant physiological mechanisms, the methods of amelioration, and the general implications for psychobiology.

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RH 00467 (Continued)

Progress Report: Ionizing rays have been described as "energy which is not a stimulus", although Wilhelm Roentgen and other early workers soon became aware that X-rays could elicit physiologic responses under certain conditions. Unlike stimuli usually employed in the psychobiological laboratory, ionizing radiations are a pervasive form of energy penetrating living matter and delivering energy throughout all the tissues and fluids of the body. The absorption of this energy causes basic molecular changes; atoms eject orbital electrons and molecules dissociate, creating ions and radicals which alter the permeability of membranes and interrupt ongoing functions of cells. Depending on the density of the matter encountered, high energy photons may pass entirely through the organism producing a shadow that when recorded on photographic film, reveals the essential transparency of the body to this form of energy.

Our previous research (supported by RH 00068) has demonstrated that behavioral effects of radiation are dependent upon the physiological site of energy absorption and the specific test imposed upon the organism by the experimentor. When radiant energy is absorbed by peripheral receptors, intensity requirements for eliciting a response are low, latencies are brief, and orientation is the characteristic response. Immediate effects can be observed in motor responses and electrical activity of the brain. The use of brief X-ray exposures as a signal to warn animals of a subsequent shock to the paws has indicated that the probability of response is a function of the log dose rate between 0.01 and 0.40 r/sec. for 250 KV X-rays. The reaction time is less than 150 milliseconds and the total dose required to elicit the response is less than 10 milliroentgens. Tests with a narrow collimated beam directed at the head indicated that peak sensitivity is in the olfactory region. Subsequent experiments indicated that ablation of the olfactory bulbs produced a marked decrement in the frequency of arousal responses to X-rays. Current work indicates that olfactory stimulation is due to direct action of ionizing rays upon the olfactory receptors.

Several hours after exposure, rats like men and many other mammals, suffer from "radiation-sickness". In monkey and man, this syndrome consists of a brief symptom-free period of a half hour or more, followed by nausea, emesis and gastro-intestinal

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disturbances. One of the most sensitive measures of this effect in experimental animals is the conditioned aversion test. This test consists of giving the animal a distinctive solution (for example, water flavored with saccharin) which he drinks in conjunction with radiation exposure. A whole body exposure of 10-30 R of X-rays will produce a significant reduction in the rat's usual preference for saccharin-flavored water. (Control animals exposed to an equal dose of X-rays, but not while drinking saccharin-water, show no later decrement in preference for the sweetened fluid.)

Our recent experiments have demonstrated that the conditioned aversion can also be established with injections of radiomimetic drugs, emetic drugs or serum taken from irradiated donors. Serum from irradiated donors will also cause intestinal spasms in animals. This indicates that radiation exposure produces a circulating toxoid product which is responsible for radiation illness. We are attempting to identify this toxoid product with blood serum fractionation techniques.

These findings have general implications for psychobiology. Specifically, two theoretical propositions of conditioning and learning need to be re-examined. First is the Pavlovian hypothesis that any natural phenomenon chosen at will may be converted into a conditioned stimulus or signal; for example, any discriminable stimulus such as a light or sound if it precedes food on a number of occasions will acquire the capacity to elicit responses associated with feeding in the absence of food. However, our research indicates that when an animal suffers radiation illness, then conditioned avoidance responses are transferred to the gustatory and olfactory stimuli but not to the auditory or visual stimuli. Conversely, when an animal suffers peripheral pain from electric shock, the conditioned avoidance responses are transferred to auditory and visual stimuli but not to gustatory and olfactory stimuli. In other words, from the welter of stimuli in its environment the animal "selects" its cues depending upon the nature of the subsequent stress. If the stress is internal illness associated with chemical changes in the fluids of the body and monitered by internal homeostatic receptors, the animal is neurologically programmed

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to select cues from its chemical analysers (taste and smell). However, when the stress is external pain signaled by skin receptors, the animal is neurologically programmed to use cues provided by its distance receptors (sights and sounds).

Second, it is generally considered that effective learning cannot occur unless reinforcement is immediate. For example, a hungry animal will not learn a response (e.g. press a lever) unless this response is immediately followed either by primary reinforcement (e.g. food) or secondary reinforcement (e.g. a signal previously paired with food).

But our recent research has demonstrated that gustatory aversions are readily established by conditionally pairing a distinctive flavor with a noxious drug, even when the drug injections are delayed for an hour or more. Similarly, vitamin-deficient animals will display an increased preference for a non-nutritive fluid if the ingestion of this fluid is followed by delayed vitamin injections. Apparently immediate reinforcement is not a general requisite for all learning.

Since the ultimate effects of ingested materials are often delayed, effective gustatory-visceral associations would necessarily need to span longer temporal intervals than other receptor-cutaneous connections. Presumably specialization has occurred in the brain mechanisms which serves these afferent modalities. Thus the neural mechanisms of learning and memory may differ depending upon specific sensory modalities used to signal the animal and to reinforce its responses.

Significance of Research: This research is directly related to radiation hazards. The immediate response of olfactory receptors may be indicative of damage to structures common to all cells of the body, such as permeability changes in cellular membranes which could disrupt life functions of any cells. Moreover, injections of blood serum from irradiated donors produces toxin-like reactions in non-exposed recipient animals. Thus energy absorption by the tissues of the body during exposure with its attendant formations of ions and radicals may be turning normal metabolities into stable toxic compounds. Isolation of the specific components in the serum from exposed donors

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RH 00467 (Continued)

responsible for this effect could be the primary step in designing new therapeutic treatment for victims of radiation exposure.

Publications:
Garcia, J. and Koelling, R. A. "A Comparison of Aversions Induced by X-Rays, Toxins, and Drugs in the Rat." Radiation Research, Supplement 7, 439-450, 1967.

RH 00473-02

PERCEPTION OF IONIZING RADIATION BY SENSORY SYSTEMS

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Research Objectives: This research proposes to obtain information on two vital questions:

1. Can low-level ionizing radiation have an effect on the central nervous system?

2. Can low-level ionizing radiation serve as a stimulus to synchronize physiological rhythms?

Progress Report: Due to the nature of this project, which requires that animals be free of all disturbances in a controlled environment for extended periods of time, we have had to use a species which requires minimal care in the form of food and water requirements. Most important from a practical point of view, since other investigators use the X-ray machine, we also wanted an animal which exhibits minimal waste excretion. This would allow the animals to be undisturbed for two to three week periods and still not foul the X-ray room. Such an animal is a species of gerbil, Meriones unquiculatus. As nothing was known of this animal's rhythmic characteristics, these were the first parameters which we determined. We have followed patterns of total body activity as our test rhythm.

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This species is nocturnal: more than 90% of its activity occurs during the dark phase of its day. After its activity has been randomized by extended exposure to total darkness, an alternating light and dark regime will cause the onset of activity to be synchronized to the dark phase of a daily light cycle. This occurs in 5 to 8 days. Inversion of the light regime leads to inversion of the activity regime. In constant darkness the animals exhibit "free running" periods of 23 to 25 hours in length. In view of current theories in this area, all of these experiments indicate that the gerbil is using light (or the lack of light) as a stimulus to synchronize its daily patterns of activity. One heretofore unanswered question in rhythm research has been: in total darkness do the animal's rhythms persist because of an internal clock mechanism, or does the animal receive some other cue from the ambient environment which synchronizes rhythmic events even in the absence of light? Determining the answer to this question is one of our major abjectives.

In preliminary experiments, we have seen that, in the absence of light, gerbils can use a low-level ionizing radiation stimulus to synchronize rhythms of total body activity. We are now in the process of substantiating this finding, and, having found that animals can use this stimulus, extending our research to determine if animals do indeed use ionizing radiation to synchronize physiological rhythms.

Significance of Research: If low-level ionizing radiation is necessary as an environmental cue for normal physiological processes, this work will have far-reaching implications. Having an effect on the Central Nervous System involves the basic system necessary to integrate body functions. It is very easy to see that increasing the low levels of radiation, such as cosmic radiation, etc., which normally impinge on the earth's inhabitants, will upset the processes which are basic to normal life. This of course is in addition to the other better known hazards of excessive radiation.

Publications:

Mizell, S., Barnes, C. D. and Daugherty, J. "Activity Rhythms in the Gerbil." Anatomical Record 160:394, 1968.

Mizell, S., Barnes, C. D. and Daugherty, J. "Effect of Ionizing Radiation on Activity Rhythms in the Gerbil." Proceedings of Vth International Congress on Photobiology, Hanover, New Hampshire, pg. 114, 1968.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00482-03	CYSTEAMINE AND X-RAYS PRENATALLY: BEHAVIOR OF THE RAT	JOAN M. ROBERTS, PH.D. DEPT. OF ORAL BIOLOGY UNIVERSITY OF DETROIT DETROIT, MICHIGAN

Research Objectives: The purpose of this project is to further study the effects on the mental and emotional functioning of offspring whose mothers were exposed to ionizing radiation during pregnancy. We are also continuing our work with a drug, cysteamine, that we have shown to protect these offspring against central nervous system damage that often follows radiation exposure.

Progress Report: We have demonstrated that giving cysteamine prior to irradiation of the pregnant rat markedly protects the developing offspring. Offspring whose mothers were not given cysteamine were significantly retarded in the development of responses involving motor maturation. These animals also were significantly slower in learning to traverse a maze.

Weight curves were also plotted for cysteamine-protected and unprotected groups of offspring and they indicate that growth is significantly retarded in those whose mothers were irradiated but not treated with cysteamine.

Our more recent work has shown that cysteamine is more protective when given early in pregnancy. Those animals receiving the drug (cysteamine + irradiation) at later stages do not fare as well, with those treated during the second trimester being the least protected. Also, as radiation dose increases, this protective effect is less apparent. We are, therefore, going to increase the cysteamine dosage to see whether the drug can then be used at more lethal levels of radiation without the drug itself being toxic to the animals.

Measures of learning ability and neural functioning that we have not previously studied will also be evaluated in the present project. This will include the re-learning of previously learned material and learning to discriminate tones.

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We also intend to further investigate the effects of stress on the adrenocortical system of the newborn. From previous data and data to be collected in this project, we hope to get some evidence of what is happening to the endocrine system in the developing mammal exposed to radiation that so profoundly affects his later behavior, growth, and development. Administration of the protective drug after radiation exposure is also being studied to determine whether the damaging effects of radiation can be prevented or at least lessened in the developing young.

Significance of Research: This project will help us to acquire a more comprehensive view of the area of prenatal irradiation, radiation protection of the fetus by chemical means, and the effects of prenatal stress (e.g., irradiation) on postnatal behavior and development. Experimental work with the pregnant rat will aid us in approaching the problem of potential radiation hazards in our own environment.

Publications: None

RH 00487-02 CYTOGENETICS OF IRRADIATED MALE MICE

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Research Objectives: The objective of this project is to determine the extent of permanent radiation damage to spermatogonial stem cells in the mammalian testis after exposure to ionizing radiation. In this study, cytologically visible major chromosome aberrations, particularly translocations, are the chief indicators of damage and are readily demonstrated in the offspring of irradiated male mice.

Progress Report: Prior to the publication of the early results from this project, there had been no tangible evidence that permanent genetic damage results from the exposure of generative stem cells in the mammalian testis to ionizing radiation. This study has now shown (a) that drastic germ cell damage is caused; (b) that the damage is clearly visible under the microscope since it consists in large part of

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gross aberrations of the chromosome system; (c) that the damage is permanent, causing irradiated males to generate abnormal germ cells and to transmit aberrations to their offspring throughout the remainder of their reproductive life; and (d) that the offspring which results from translocation-bearing germ cells may be completely sterile or semi-sterile, in the latter case producing a further generation of carriers of the abnormality. The frequency of such aberrations after several radiation dosages below 1000 R is currently being determined. It is notable that large numbers of animals must be used, and large numbers generated and tested, in this program.

It is therefore best carried out with the use of the laboratory mouse, from which extrapolations can be made to human beings. It is also notable that The Jackson Laboratory is an ideal locale for such work, since it uniquely provides both the facilities and the animal resources which are required.

Significance of Research: It has long been assumed that after testicular irradiation, male mammals (and men in particular) might refrain from procreation for a period of two or three months, and thereafter sire offspring which would be free of radiation-induced mutations and aberrations. The present study conclusively shows this assumption to be wrong, since radiation damage to testicular stem cells is permanent and permanently transmissible. It strongly points up the necessity for caution and protection in medical diagnostic and therapeutic procedures, as well as in accidental irradiation from nuclear warfare and atomic devices.

The earlier findings in this project greatly influenced two international reports on genetic risks to man (United Nations Scientific Commission on the Effects of Atomic Radiation, and International Commission on Radiation Protection), according to a reviewer of a paper submitted for publication in the Canadian Journal of Genetics and Cytology. The continuation of this study will provide a highly accurate determination of testicular damage from low doses of irradiation such as may be anticipated for human beings in the atomic age.

Publications: None

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00496-01	EFFECT OF RADIATION ON AMINO ACID TRANSPORT	ELLEN G. ARCHER, PH.D. DIV. OF NUCLEAR MEDICINE WASHINGTON UNIVERSITY ST. LOUIS, MISSOURI

Research Objectives: The objectives of this research were 1) to evaluate the sensitivities to ionizing radiation of certain functions of the membranes enclosing biological cells in relation to other processes essential to life carried out inside the cells such as the synthesis of proteins and nucleic acids and the production of energy needed to maintain life; 2) to compare the radiosensitivities of different systems located in cell membranes which serve to facilitate the entry into cells of amino acids, essential building blocks for the synthesis of proteins. It was thought that radiation might provide a useful tool for the study of certain membrane processes in isolation, others having been rendered inoperative by radiation.

Progress Report:
In Vitro Irradiation of Ehrlich Ascites Cells

Ehrlich ascites carcinoma cells, a type of tumor cell which grows free in fluid which collects in the abdomens of injected mice, are more suitable for the study of cell membrane functions, such as the transport of amino acids into the cell, than are solid tumors, in which the membranes of individual cells are cemented together. Ehrlich cells were removed from mice, suspended in warm medium containing the desired concentrations of sodium and other dissolved substances and having the normal acidity of body fluids, pH 7.4, and placed close to a powerful source of penetrating gamma radiation from ^{60}cobalt for varied times. The cells were then removed from the radiation source and added to appropriate medium containing a specified amino acid one of whose carbon atoms was radioactive; sometimes other amino acids, not containing radioactive carbon, were present in concentrations previously shown to effectively inhibit transport through the cell membrane of the particular radioactive or "labeled" amino acid being studied by transport routes other than the one it was desired to

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study. The unlabeled amino acids compete with the labeled amino acid for binding to the systems in the cell membranes which mediate entry into the cell. After incubation at 37° for eactly one minute, the soluble intracellular contents of the cells were extracted with acid and counted for radioactivity. The radioactivity inside the cells is a measure of the amount of labeled amino acid which has passed from the external medium into the cells during the time of incubation. Results were compared with those obtained using portions of the same cell suspension which had not been placed near the source of gamma radiation, i.e., "control" cells.

Uptake of amino acids into the intracellular fluid of ascites cells by the membrane transport systems designated "A" and "ASC", as well as the intracellular content of potassium, was lowered to a similar degree after equal doses of gamma radiation. All three of these membrane processes require energy supplied from metabolic processes within the cell for operation. The amino acid transport systems designated "L" and "$L^{o,+}$" are much less sensitive to the same doses of radiation; these systems apparently do not require energy. From consideration of the observation of others that the highest dose of radiation used does not markedly diminish the availability of cellular energy and the failure to demonstrate generalized increased "leakiness" of the cell membranes after irradiation it was suggested that radiation interferes with transport of amino acids by the A and ASC routes and with potassium transport by inactivating a hypothetical large molecule or complex of large molecules closely associated with the cell membrane which serves to channel energy into membrane transport systems.

The differences in radiosensitivity between the different transport systems studied were not sufficient to provide a practical means of selective inactivation of all but one of those of a group of transport systems used by a particular amino acid.

At the time this work was begun, there were suggestions in the literature that the cell membrane might be the site of the "initial lesion" after irradiation, i.e., the area of the cell whose molecular structure is first disturbed by ionizing radia-

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tion. If this is so, the "initial lesion" is quickly repaired and the disturbance transferred to the area of the nucleus, where damage due to radiation is much more profound. The membrane processes we studied were inhibited only by doses of gamma radiation several times that needed to stop cells from dividing. Interference with cell division after irradiation is closely linked to effects on the replication of deoxyribonucleic acid in the nucleus.

Amino Acid Transport in Lactobacillus casei

Two amino acid transport systems in this micro-organism were partially characterized. One has greater affinity for amino acids with short sidechains, such as glycine, L-alanine and α-aminoisobutyric acid than for amino acids with long sidechains, such as L-leucine and 1-aminocyclopentane carboxylic acid. The affinity of the second system is roughly opposite that of the first. Both systems require energy and are affected by the acidity of the medium.

Significance of Research: Our results support the concept that the cellular nucleus is the prime target of ionizing radiation and is of central importance in explaining the filling of cells by radiation. If the "initial lesion" is in the cell membrane, it must be quickly repaired, as we were not able to observe profound effects of radiation on certain transport functions of the membrane, except at very high doses.

Publications:

Christensen, H. N., Liang, M. and Archer, E. G. "A Distinct Na^+-requiring Transport System for Alanine, Serine, Cysteine, and Similar Amino Acids." The Journal of Biological Chemistry, Vol. 242, No. 22, pp. 5237-5246, November 25, 1967.

Archer, E. G., "Inactivation of Amino Acid Transport Systems in Ehrlich Ascites Carcinoma Cells by Cobalt-60 Gamma Radiation." Radiation Research, Vol. 35, No. 1, pp. 109-122, July 1968.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00499-02	EFFECTS OF PROTON IRRADIATION	JOHN J. GHIDONI, M.D. DEPT. OF PATHOLOGY BAYLOR UNIVERSITY COLLEGE OF MEDICINE HOUSTON, TEXAS

Research Objectives: Rhesus monkeys were whole-body irradiated with protons at several different, discrete energy levels (32, 55, 138, 400 Mev and 2.3 Bev) and studied electron microscopically at intervals of 10 minutes, 6, 12, 18, 24, 36, and 48 hours following irradiation. Indications of the mode of interaction between proton particle beams and subcellular components (cellular organelles) were sought in the ultrastructural alterations in liver and small intestine.

Progress Report: Depth-dose distributions of energy given off by protons were qualitatively demonstrated in primate liver. The animals were irradiated with 7,000 rads (surface dose) of 32 Mev protons and 6,000 rads (surface dose) of 55 Mev protons. The hemorrhagic lesions produced by protons of both energies were similar. The higher-energy proton beam produced a lesion at a greater depth (32 Mev proton, 0.5 to 3 mm; 55 Mev proton, 18 to 20.5 mm). Their respective locations were compatible with the calculated positions for the Bragg peaks. Cell death as well as cell damage characterized irradiated liver. Pooling of red blood cells in sinusoids was commonly present. During the 36- to 48-hour post-irradiation period the most prominent electron microscopic features of the hepatocytes were the discrete, segmental membrane alterations which were found in mitochondria, rough and smooth endoplasmic reticulum, and microbodies. No cellular participation in the formation of the lesions or cellular response to the submicroscopic lesions was evident. Mitochondria protruding into vacuoles showed loss of matrix. The remarkable increase in the shrunken, dark cell population in the irradiated portions of liver and the elevated serum enzyme levels were considered to be supporting evidence for the ultrastructural observations of membrane alterations. Blebs (balloon-like enlargements of distal portions of hepatocytic microvilli) were a prominent feature

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during the first day following irradiation. The blebs ranged widely in size, and many protruded into the sinusoidal lumen. A few micrographs showed sinusoidal lumens to be filled with bleb-like structures. Such bleb formation suggests a possible mechanistic explanation for hepatic congestion following high levels of irradiation. The morphologic alterations of proton-irradiated small intestine are similar to those reported for studies that employed electromagnetic irradiation (X-ray) of primates and other species.

Significance of Research: An understanding of the complex interactions between radiations and biological specimens is needed in this era of nuclear technology. The general population is subjected to low-level, background radiation. Irradiation to vital organs can accompany radiotherapy. High dosages of radiation of vital organs can also result from "criticality" accidents and from modern warfare. Applications of radioactive isotopes both in diagnosis and in therapy are rapidly increasing. The more precise our knowledge is concerning the mechanisms of action of radiations on cells, the more rational can our approach be to the investigation of means for preventing or diminishing the untoward effects of irradiation. Radiations in space present a potential hazard to astronauts. Most investigations in radiobiology have dealt with the effects of electromagnetic irradiation (X-rays and gamma rays). Medical use of particulate radiation for therapy is now a rapidly expanding field and presents a very down-to-earth requirement for new knowledge.

Publications: None

RH 00501-14 — MODIFICATION OF BACTERIAL CONJUGATION BY RADIATIONS

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PHILADELPHIA, PENNSYLVANIA

Research Objectives: Bacterial conjugation (union, chromosome transfer, genetic recombination and segregation) may be modified by radiation applied to either parent at various stages of the process. Our objectives are two: to study in detail and

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breadth the various steps of conjugation and to examine the effects of radiation on chromosomes using a wide variety of endpoints for the detection of radiation damage and its repair.

Progress Report: Sexuality is the process by which genetic information from two parents is mixed together, recombined, and assorted to give progeny differing from either parent in one or more genetic characteristics. All higher organisms experience sexuality but often differ in the details of the various steps. For the past five years we have studied conjugation in the bacterium Escherichia coli K-12. With this rapidly growing organism it is possible to carry out studies with large populations which allow a more detailed understanding of the associated processes.

During conjugation male and female bacterial cells come into physical contact and the male parent transfers chromosomal material (genes) in a sequential fashion into the female. The transferred male chromosomal material comes into at least partial juxtaposition with a female chromosome, similar genes being contiguous (pairing). In an unknown way genetic material is exchanged between the two chromosomes and thereby a new, recombinant chromosome is produced which contains genetic information from both parents. With subsequent cellular growth and division this new chromosome may be sorted out from the residual genetic material of the female parent.

We have studied these steps and the overall process primarily by irradiating either parent or the fertilized female (the zygote) at various times before, during, or after conjugation. A wide spectrum of radiations has been used in a comparative way (X-rays, alpha particles, P^{32} decays, ultraviolet and infrared); many of the effects observed are qualitatively similar for different radiations when properly normalized. Irradiation of the male prior to mating reduces the probability of transfer of male chromosomes, the reduction increasing with radiation dosage. The ability of cells to form a mating union may also be impaired but steps subsequent to chromosome transfer are not greatly affected.

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However, irradiation of the female parent does not affect the transfer of male material but diminishes the overall production of recombinant cells. Furthermore in those recombinants that are formed, there is a preferential utilization of male genetic material which increases with dose. Thus by using radiations selectively recombinants may be produced which preferentially use genetic information from the non-irradiated parent. It has been possible to formulate a detailed mathematical model for the pertinent genetic processes which predicts the relative contribution of either parent as a function of radiation dose. The model and additional experiments suggest that other injurious agents, especially radiomimetic ones, may be analyzed in a similar fashion.

Radiations may also be used to study the recombinational processes which occur in the zygote (the fertilized female). Immediately after transfer of male chromosomal material into the female, the recombinational processes are greatly enhanced by irradiation of the zygote as if additional chromosomal fragmentation is produced. However, these processes become radiation resistant after approximately one cell division and we conclude that a stabilized recombinant chromosome has been produced. Thus again it is possible to use radiations as tools for the enhancement of desired genetic effects.

Little is known of the "crossing-over" events between male and female chromosomes which produce the recombinant chromosomes; we are searching for the "rules" which apply. It appears that these events are not completely random but occur in groups which are widely dispersed along the chromosome (negative interference). Furthermore a single zygote may produce several recombinant types, a finding which strongly suggests an extended persistence of the male genetic material for several cell divisions. All of these results are modified by radiation treatment.

Of particular interest are experiments both completed and in progress in which this mating system is used to differentiate between radiation effects operative directly on the genetic material (the DNA) and on other cellular components (the cytoplasm).

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Since the donor parent transfers only genetic material to the female, any radiation effects produced by irradiation of the male and transferred to the female must have been carried on the DNA. Cytoplasmic modification of radiation damage can also be studied by such transfer experiments.

The E. coli K-12 mating system is particularly useful for the study of radiation biology because of the large number of detectable radiation effects that may be used. Among those we have used are colony formation, mutations, chromosomal rearrangements, ability to form mating unions, ability of the male to transfer its chromosome into the female, speed of chromosome transfer, direction of chromosome transfer, pairing of parental chromosomes, "crossing-over" frequency between parental chromosomes, kinetics of recombinant production and number of recombinant classes produced per zygote. Furthermore many of these steps are under known genetic control and may be varied as desired. Thus this organism has the versatility to serve as a model system to study basic mechanisms which will probably be operative in more complicated systems.

Significance of Research: Biological effects of radiations can be most easily studied with microorganisms. Most studies, however, have been carried out using only cell survival as the index of radiation effect. With bacterial conjugation, it is possible to obtain many operational endpoints for radiation damage which afford much finer discrimination than previously available and that may give insight into radiation damage and repair in mammalian systems. Genetic control of radiation sensitivity and repair is now firmly established in bacteria and probably is operative in more complicated organisms. We have developed techniques that allow modification and control by radiations of the relative contributions of either parent to recombinant progeny and which provide the basis for a type of genetic engineering that could be useful in research and technology.

Publications: None

RADIATION BIO-EFFECTS

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(3) RH 00512-01	RADIATION INDUCED CHROMOSOME DAMAGE AND CELL RESPONSE	FRANK J. SOBKOWSKI, D.D.S., M.S. SCHOOL OF DENTISTRY UNIVERSITY OF CALIFORNIA LOS ANGELES, CALIFORNIA

Research Objectives: The primary objective of this project is to obtain information on the patho-physiologic processes involved in the production of late somatic effects of ionizing radiation in vivo.

Experiments are designed to simulate the temporal and physiologic conditions under which the late effects of radiation develop. The parameters to be studied include the incidence and types of radiation-induced chromosomal lesions, the kinetics of cellular and tissue regenerative phenomena and the histopathologic response of the irradiated tissue.

It is hoped that the data from this project, when correlated, will provide information that not only is presently unavailable, but that will provide a base on which additional information may be developed so that an appreciation may be gained of the mechanisms underlying the pathologic processes involved in the production of the late somatic effects of radiation.

Publications: None

(3) RH 00537-01	NEURAL DETECTION OF IONIZING RADIATIONS	DONALD J. KIMELDORF, PH.D. DEPT. OF GENERAL SCIENCE OREGON STATE UNIVERSITY CORVALLIS, OREGON

Research Objectives: There is reasonable evidence ot indicate that the nervous system can respond to very small doses of ionizing radiations. The reaction is very similar to that induced by stimuli for which specific receptor systems have been established. No receptor is known for which ionizing radiation is the adequate

RADIATION BIO-EFFECTS

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stimulus. It is possible that radiation may be as effective as a physiological stimulus for several receptor systems. Evidence for this point of view, however, is limited and the available routes for the detection of ionizing radiations have not been established. Most of this work has been done in animal species that possess highly differentiated and complexly organized nervous systems. The purpose of this study is to examine the problem of biodetection in species of lower phyla with less differentiated systems. The study of mechanisms for detection in simpler systems should provide information that is instructive in the interpretation of this phenomenon in the more complex species. The manner in which representative organisms of several phyla are informed about their own exposure will be explored in order to establish the systems that might, under certain conditions, serve usefully as biodetectors for the presence of ionizing radiations.

Publications: None

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00005-07	RADIATION EFFECTS ON FORMATION OF PROTEIN AGGREGATES	HERMAN R. HAYMOND DEPT. OF RADIOLOGY UNIVERSITY OF SOUTHERN CALIFORNIA LOS ANGELES, CALIFORNIA

Research Objectives: This investigation is concerned with the mechanism by which ionizing radiation interferes with interactions between proteins in aqueous solution, particularly interactions involving weak bonds in the formation of protein aggregates. Current work is related to the effects of radiation on interactions between antigens and antibodies.

Progress Report: A study of the effects of ionizing radiation on the interaction between beef insulin and rabbit serum antibodies-to-insulin has indicated that one of the intermediate free radicals, OH, formed by action of the radiation on water, is the principal agent for radiation damage to the antibody (gamma globulin) molecule. The yield of radiolysis is 0.15 protein molecules inactivated per 100 electron volts of radiation energy absorbed. An estimate of the reaction rate constant for the reaction between the OH free radical and the protein molecules has been obtained from studies involving other competing solutes for which reaction rate constants were previously known. The role of oxygen in the radiolytic process has been studied and there is evidence that the initial inactivation occurs without participation of oxygen, although oxygen is involved in the subsequent destruction of peptide bonds in the protein chain. A study of the role of hydrogen ion concentration indicates that some protection may be afforded the protein by aggregation in neutral solutions. In the course of this investigation a method was developed for measuring small changes in concentration of antibodies-to-insulin. The method involves formation of an antigen-antibody complex with radioactively labeled insulin and subsequent separation of the free insulin with an anion exchange resin.

Significance of Research: In the complex chain of events which occur between the initial physical absorption of radiation energy in a biological system and the

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RH 00005 (Continued)

subsequent observable effects on the organism, there are several levels which deserve investigation. This study is concerned with the effects of radiation at the chemical level, and particularly at the level of large molecules which form aggregates involving weak bonds. Studies in this area have the possibility of contributing knowledge about potential methods of altering the course of early chemical events. Methods for diminishing the effects of radiation in man would of course aid in control of radiation hazards, but any modification of radiation effects might also result in an improvement in radiation therapy techniques in treatment of cancer, because the therapist is faced with the dual problem of causing minimal injury to normal tissue while destroying malignant tissue. In addition, this study might produce basic information relating to the chemical interactions involved in formation of an antigen-antibody complex and hence possibly contribute to our understanding of immune diseases or immunologic responses to disease.

Publications: None

RH 00042-07

COMPOUNDS TO PROMOTE EXCRETION OF RADIOISOTOPES

WILLIAM H. STRAIN
UNIVERSITY OF ROCHESTER
ROCHESTER, NEW YORK

Research Objectives: Compounds are being developed to promote the excretion of radioisotopes. These preparations will find application in reducing the danger from radioactivity received by persons exposed to radionuclides. Thus, the hazards from nuclear accidents and bombs will be reduced, and long-lived radioisotopes may be used more safely for diagnosis and therapy in medicine. We are synthesizing and studying organic complexes of a variety of elements.

Progress Report: Our work has been divided into several major areas. We have developed hair analysis as a means of monitoring the accumulation of radioisotopes in experimental animals, and our method has been applied by others to man. We have prepared and purchased a variety of organic complexes of manganese, mercury, and zinc,

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and have studied these in animals as a means of promoting the excretion of corresponding long-lived radioisotopes. We have developed a technique of using a whole body counter as a means of measuring the excretion of radioisotopes by small animals. We are now entering the stage of clinical application of our preparations to the promotion of radioisotope excretion in man. In reducing our work to human use, we are now forced to consider the requirements of the Food and Drug Administration. As part of our overall program, we have prepared a monograph on radiologic diagnostic agents.

We began our work by investigating the composition of hair as a measure of element content of the body. We felt that radioisotope retention would be greater in deficiency states. We found that radioisotopes accumulated in hair, and suggested that hair analysis would furnish a method of monitoring fallout. This work was based on studies conducted with strontium-90 in Long-Evans rats, and was immediately confirmed by others working with beagles and man. Almost all radioisotopes do accumulate in hair, so that the method has wide application.

A variety of EDTA derivatives have been prepared as agents to sweep out radioisotopes. We synthesized manganese disodium EDTA and zinc disodium EDTA, and then discovered that these compounds are widely used as fertilizer ingredients and are readily available. Both compounds are very satisfactory in promoting the excretion of the corresponding long-lived radioisotopes from rats. Presumably, they will be equally satisfactory in man, since the correlation of results between rats and man is good. While our work was in progress, Catsch in Germany showed that cobalt derivatives of EDTA promoted the excretion of radiocobalt from rats. These EDTA derivatives are particularly valuable as protective agents in case of nuclear accidents from bombs or reactor power plants.

We have compared the efficiency of several organic mercury compounds in promoting the excretion of chlormerodrin-Hg-203. Chlormerodrin, the corresponding cold compound, is the best agent for sweeping out the hot preparation. This work is important for

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the initiation of clinical studies. Since chlormerodrin is an established drug and FDA clearance is not needed, this preparation may be tested immediately. We propose to use cold chlormerodrin to sweep out chlormerodrin-Hg-203 after brain scanning. The radiomercury derivative is far cheaper than the more widely used technetium-99m and gives more accurate brain scans.

We have prepared a monograph on radiologic diagnostic agents and were able to obtain wide distribution at a minimum of expense. Our monograph was published as a supplement to Medical Radiography and Photography, a publication of Eastman Kodak Company in 1964, and was distributed to over 70,000 radiologists and investigators.

Significance of Research: We have developed a variety of compounds to promote the excretion of radioisotopes and have other under study. Our compounds are organic complexes of the cold form of the element corresponding to the radioisotope. We expect our preparations will be useful in the treatment of people exposed to radionuclides accidentally released. These agents will find application also in diagnostic radiology by promoting the excretion of inexpensive, long-lived radioisotopes. At present, short-lived radioelements are widely used for diagnosis, but are costly. We believe we can greatly reduce the cost of brain scans for tumors and strokes by using cold chlormerodrin to sweep out hot chlormerodrin-Hg-203.

Important discoveries were made early in our work. We found that radiozinc and a number of other radioisotopes accumklated in vascular tissue. This retention suggested that zinc is important to the maintenance of the integrity of the blood vessels. Since we had also discovered in other work that zinc deficiency is common in atherosclerosis, we have successfully developed an inexpensive zinc therapy for the treatment of intermittent claudication. We suspect that the beneficial effect of zinc may be due to promotion of healing. These investigations on therapy for atherosclerosis and the promotion of healing are being sponsored by other funds, but we shall continue to credit RH 00042 for the basic observations.

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Publications:

Strain, W. H., Rob, Charles, G., Pories, W. J., Hennessen, J. A., Barker, W., Montoya, John, "Zinc-Binding Capacity of Serum." Abstract K-6, Journal of Nuclear Medicine, Vol. 8, 299-300, April 1967.

Strain, W. H., Pories, W. J., Zaresky, S. A., Dattilo, E., "Health Hazards from ^{65}Zn Pollution." Transactions of the American Nuclear Society, Vol. 11, 60-61, June 1968.

Strain, W. H., Peer, Richard M., Thomas, M. R., Gass,A. P., Pories, W. J., "Promotion of ^{203}Hg-Chlormerodrin Excretion by Cold Chlormerodrin." Journal Nuclear Medicine, Vol. 9: 373, June 1968.

Strain, W. H., Rob, Charles G., Pories, W. J., Hennessen, John A., Montoya, John, "Zinc-Binding Capacity and Zinc Levels of Serum." Journal Nuclear Medicine, Vol. 9: 352, June 1968.

Strain, W. H., Peer, R. M., Thomas, M. R., Gass, A. P., Pories, W. J., "Promotion of Radiomercury Excretion." Abstract, Conference Internationale sur l'utilisation des Isotopes radioactifs en pharmacologie, Geneva, p.67, September 20-23, 1967.

Strain, W. H., Peer R. M., Thomas, M. R., Gass, A. P., Pories, W. J., "Gonadal Retention of Radioselenium." Abstract, Conference Internationale sur l'utilisation des Isotopes radioactifs en pharmacologie, Geneva, p. 67, September 20-23, 1967.

Strain, W. H., Pories, W. J., Rob, C. G., Henzel, J. H., Hennessen, J. A., Plecha, F. R., "Trace Elements and Wound Healing." Paper presented at the University of Missouri's First Annual Conference on Trace Substances in Environmental Health, Memorial Union, University of Missouri, Columbia, Missouri, July 10, 1967.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00089-05	X-RADIATION PROTECTION BY CHEMICAL AGENTS IN GUINEA PIGS	RICHARD WAGNER, M.D. DEPT. OF PEDIATRICS TUFTS UNIVERSITY SCHOOL OF MEDICINE BOSTON, MASSACHUSETTS

Research Objectives: This research is a variety of studies on irradiated guinea pigs preprotected by the injection of ATP or RNA. Irradiated guinea pigs preprotected by these agents and control irradiated animals will be studied for: 1. Studies on breeding performance, 2. measurements of ATP concentrations in the whole blood, 3. ATP concentrations in the leukocytes with time, 4. ATP concentrations in the mitochondrian fraction of leukocytes, 5. an analysis of the purine base composition of the leukocytes, and 6. extended studies on the protective effect, using pure lines of guinea pigs. The irradiation will be from 250 to 295 R total body irradiation using an X-ray machine 260 KV, 15MA, 1mm. Alum., 0.5 mm. copper, 50 cm. distance MVL 1.2 mm copper. The LD_{50} for the guinea pig is 200 R.

Publications:

Wagner, R. and Silverman, E. C. "Chemical Protection Against X-Radiation in the Guinea Pig, I. Radioprotective Action of RNA and ATP." International Journal of Radiation Biology, Vol. 12, No. 2, 1967.

RH 00095-09	INACTIVATION OF ENZYMES BY IONIZING RADIATION	EVELYN SLOBODIAN, PH.D. DEPT. OF RADIOLOGY NEW YORK UNIVERSITY MEDICAL CENTER NEW YORK, NEW YORK

Research Objectives: The goal of medical radiation research is to realize the potential of radiation techniques in the fight against disease and to minimize attendant hazards. Towards this end, it is necessary to inquire into the basic mechanisms of radiation effects. Our laboratory has been studying the irradiation of ribonuclease, one of the enzymes which may be involved in the control of growth

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and reproduction. Our objective is a sophisticated understanding of how irradiation damages the enzyme molecule, and how such damages correlate to impairment of enzyme function.

Progress Report: Ribonuclease is an attractive enzyme to study; it is stable, easily purified, and amenable to laboratory experimentation. Most important, intensive studies of the last 15 years have elucidated its structure and defined the types of changes which affect its activity.

The enzyme is a chain of 124 amino acids, coiled into an almost spherical shape, and cross-linked internally by four disulfide bridges. The enzyme function depends on the integrity of the "active site", which includes two histidine residues, one from the beginning of the chain (position 12), and one from the end (119). Two lysines (positions 7 and 41) have also been implicated. Maintaining this specific arrangement requires two of the four internal disulfide bridges, as well as reinforcement by numerous weaker internal bonds. The architecture of the molecule permits many modifications in "unimportant" locations; such changes do not affect the enzymic activity.

With this profile in mind, we can now discuss our work on the irradiation of ribonuclease.

All irradiations were carried out on solutions of enzyme in water. Samples were irradiated with gamma rays from a Co-60 machine, at doses of 30,000 R to 150,000 R, to give a series of samples with varied amounts of residual enzymic activity. This series was ideally suited for a systematic study of progressive changes in physical and chemical characteristics compared to progressive loss of enzyme function.

The most noticeable and easily measurable effect of irradiation was on the physical shape of the enzyme molecule, as measured by well-established analytical techniques. Thus, the viscosity of a molecule in solution is much higher if the molecule is

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elongated than if the molecule is a sphere. Viscosity measurements of irradiated samples and intact samples showed that irradiation had, progressively, caused a three-fold elongation of the molecule. Since it is known that the coiled structure of normal enzyme is reinforced by internal bonds, it appeared likely that many of these bonds were broken upon irradiation, thereby permitting the molecule to elongate. Most of the internal bonds are "weak" bonds, referred to as hydrogen bonds or hydrophobic bonds. Their presence is readily demonstrated by experiments in which the enzyme is incubated with tritiated (radioactive) water, T_2O. The bonded hydrogens of the molecule are not freely accessible to the medium, and they exchange very slowly, if at all, with the Tritium in the T_2O. On the other hand, if the molecule is "unfolded" (ex. by heat), the H bonds are broken; these H's then exchange readily with Tritium, and the molecule becomes "labeled" with radioactivity. This labeled protein re-exchanges Tritium for Hydrogen if it is reincubated with ordinary water (H_2O). By studying the Tritium-Hydrogen "interchange" behavior of irradiated samples, it was shown that there was, indeed, a progressive unfolding of the molecule with increased dose. The most highly irradiated sample (with the maximum viscosity and least enzymic activity) behaved like an open chain, linear molecule. Further evidence came from studies of three H bonded tyrosines which do not ionize freely in native enzyme. On irradiation the H bonds were broken and the three tyrosines ionized normally.

When the magnitude of these various physical changes was plotted in comparison to loss of enzymic activity, it was seen that there was a striking coincidence between the rate of loss of activity and the rate of "unfolding" of the molecule.

In contrast to the above findings, chemical analyses of irradiated samples revealed only limited changes in the covalent structure of the molecule. Thus, amino acid analyses of highly inactivated enzyme samples showed that only five or six amino acids of the 124 had been destroyed. However, even these limited damages might be meaningful since one histidine residue was among those amino acids destroyed, as well as one lysine. As it is known that two of the four histidines in ribonuclease are in the "active site", it is tempting to speculate that, statistically, there was a very

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good chance that inactivation was caused, in part, by direct damage to histidine residues in the active site of the enzyme. In another type of analysis, it was proved that irradiation had caused the rupture of at least one disulfide bridge in the molecule. Again, this bridge might be one of the two necessary for activity.

It is important to emphasize that amino acid analyses are not sensitive enough to reveal trace amounts of damages. There can be no doubt that irradiation causes chemical lesions in the molecule; otherwise, the "unfolded" molecule would simply refold after irradiation, and the radiation effect would be reversible. For example, ribonuclease can be "unfolded" by moderate heating, or by certain solvents, but upon cooling or removal of solvent, the molecule refolds to its active configuration. The fact that irradiated enzyme is permanently inactivated indicates that the physical unfolding of the molecule is accompanied by definite chemical damages which hinder any return to an active configuration.

On the other hand, some radiation induced changes do not affect enzymic activity at all. This conclusion is based on earlier chromatographic studies which showed that irradiated samples contained altered molecules which were still enzymically active.

Possibly the most important concept in current radiation research is the awareness that, in irradiations of aqueous (water) systems, the primary effect is the production of very reactive, short-lived free radicals ($H\cdot$, e_{aq}^-, $OH\cdot$) from water; these radicals attack the solute (ex. enzyme), causing the observed "damages". Evidence for participation of the reducing radicals $H\cdot$ and e_{aq}^- has been obtained from the finding of two free SH groups (from disulfide bridge rupture) in irradiated ribonuclease. However, both our work and the work of others suggest that the OH radical may be the most important intermediate in the radiation effect. For example, treatment of ribonuclease with OH radicals from a chemical source ($Fe^{+2} + H_2O_2$) gave, as did irradiation, partially inactivated samples which contained enzymically active altered molecules. Because of the dramatic implications of these findings, we are now studying the details of the reaction between chemically produced OH radicals and

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ribonuclease, and plan to analyze such inactivated samples by the same methods applied to irradiated samples.

If chemically generated OH radicals do indeed mimic the radiation effect in major respects, the radiation researcher will have a powerful and safe new laboratory tool with which to work.

Significance of Research: Our research has demonstrated that the radiation effect on ribonuclease is very complex, comprising both chemical and physical damages to the molecule; some of these are irrelevant to enzyme activity, while others destroy the activity of the enzyme. Present evidence indicates that the radiation effect is mediated through reactive free radicals formed from water. Since every biological system must be regarded as essentially an aqueous medium, it is clear that research should be addressed to the study of these free radicals and their effects. Through such work, we can hope to acquire additional knowledge of means to enhance or minimize radiation effects.

Publications: No additional publications

RH 00098-08

ENZYMATIC AND ESR STUDIES OF RADIATION DAMAGE

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DEPT. OF BIOCHEMISTRY
NORSK HYDRO'S INSTITUTE FOR CANCER
OSLO, NORWAY

Research Objectives: The long-term goal of this work is to increase our understanding of how radiation-chemical processes occur in cells and how they can be modified. In order to elucidate the fundamental mechanisms responsible for biological radiation damage and repair, important bio-molecules are irradiated, individually and in combination with modifying substances, and the radiation changes are followed with chemical and physical methods. The results are used to interpret experiments carried out on simple biological systems such as bacteria and red blood cells.

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RH 00098
(Continued)

Progress Report: Radiation damage to mammalian tissues is believed to be due to radiation-induced changes of cellular macromolecules such as the genetic substance, DNA, and enzymes and other proteins. In mammalian cells, these substances are present in an exceedingly complex architecture. It is therefore necessary to study the radiation changes occurring when these biomolecules are irradiated in a pure chemical state.

In the study of radiation damage to macromolecules in the dry state, we have extensively used electron spin resonance (ESR) spectroscopy, a physical method which detects unpaired electrons. This method permits measurements to be made on substances in the dry state. Information can thus be obtained on the processes occurring in the interval between the absorption of radiation energy and the permanent chemical changes which are responsible for the loss of biological activity. A useful approach has been to irradiate the substances at very low temperatures and then observe the changes in the qualitative ESR spectra when the samples are subsequently heat-treated. By using this procedure, a kind of slow-motion picture has been obtained of events that occur rapidly when the substances are irradiated at room temperature. The results demonstrate that in irradiated substances a series of secondary radical reactions occur. Evidence has been obtained that these reactions involve primarily intermolecular processes. It has been found that the biomolecules may be partly protected in the dry state, by the presence of other compounds which may intercept the intermolecular radiation reactions.

The radiation damage to biomolecules frequently involves the loss of a hydrogen atom. Such a damage may be repaired by a hydrogen transfer reaction, whereby a second compound donates a hydrogen atom to the damaged biomolecule. The mechanism of such reactions has been studied extensively with ESR technique, both in dry mixtures and in frozen aqueous solutions. As hydrogen donors, various sulfhydryl compounds were used, substances which are known to play an important role in living tissues. More importantly, it was found that also another type of normally occurring cellular constituents can repair radiation damage by hydrogen transfer, namely reduced pyridine coenzymes. In experiments *in vitro* such compounds protected enzymes

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even more effectively than sulfhydryl compounds. The well-known fact that the radiation sensitivity of tissue is strongly reduced in the absence of oxygen may be related to the finding that the intracellular concentration of reduced pyridine nucleotides increases strongly under such conditions.

The mechanism of radiation inactivation of enzymes is of interest, since all metabolic processes are regulated by the activity of such biocatalysts. Since interpretation of enzyme experiments must be based on knowledge of the reaction kinetics, we have studied the kinetics of inactivation of enzymes by ionizing radiation under different experimental conditions. It has been shown how it is possible, under certain conditions, to identify the water radicals responsible for inactivation of enzymes in solution on the basis of competition experiments.

An important group of enzymes which need sulfhydryl groups to be active has been particularly studied. It was found that such enzymes can be strongly and specifically protected by a chemical blocking of their active sulfhydryl groups. This protection mechanism is the most efficient so far observed.

The effect of radiation on the properties of allosteric enzymes which play an important role in the regulation of metabolic processes, had previously not been studied. It was found that in the case of two important regulatory enzymes involved in the control of the nucleic acid - and carbohydrate metabolism respectively, the regulatory function was more sensitive to radiation than the catalytic function.

The radiation sensitivity of bacteria is known to depend, among other factors, on the base composition of the genetic material, DNA. In attempts to throw light on the reason for this relationship we have studied the formation of radiation-induced radicals in the constituents of DNA. The yield and stability of radicals formed in the different constituents seem to offer a reasonable explanation of the biological observations.

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Attempts have been made to obtain information on the extent and the mechanism of the indirect effect in bacterial cells (E. coli B), by irradiating the bacteria in the absence and presence of different compounds, and comparing the radiation sensitivity of the cells in liquid suspension at 0°C and at frozen suspension at low temperatures. The results indicate that in the absence of oxygen the direct and indirect action contribute equally to the lethal action of the radiation, and that the OH radicals are the most important water radicals involved in the indirect action.

Radiation damage to membrane structures is believed to be an important factor in the mechanism of biological radiation damage. We have studied the radiation damage to the membranes of red blood cells since the effect of this damage can be readily measured and since it is possible to isolate the membranes in pure form and to study the radiation-induced changes in their chemical structure. It was found that radiation damage to these membranes is associated with oxidation of some of their sulfhydryl groups to disulfide groups and that metabolizing red cells have a mechanism which permits them to reduce such disulfides and partially repair the damage to the membrane function.

Significance of Research: It is believed that the results obtained have contributed to our knowledge of the mechanism whereby DNA, enzymes and proteins are inactivated by radiation, and how, under certain conditions, the damage can be prevented or repaired. Such knowledge is considered essential as a basis for developing improved procedures for therapeutic application of ionizing radiation and for protective procedures against undesired radiation.

Publications:

Sanner, T., Henriksen, T. and Pihl, A. "Transfer of Radiation Energy Between Different Solutes in Frozen Aqueous Solutions." Radiation Research 32, 463-474, 1967.

Henriksen, T. "Effect of Oxygen on Radiation-Induced Free Radicals in Proteins." Radiation Research 32, 892-904, 1967.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00098 (Continued)	Henriksen, T. "Effect of Crystal Dimensions on the Yield of Radiation Induced Radicals in Organic Substances." Acta Chemica Scandinavica 20, 2898-2900, 1966.	
	Copeland, E. S. and Sanner, T. "Migration of Radiation-Induced ESR Centers in Protein Mixtures." Book of Abstracts, 3rd Intern. Congr. Radiation Res. Cortina D'Ampezzo, p. 59, Tipografia Regionale, Roma, 1966.	
	Henriksen, T. and Sanner, T. "Molecular Interactions in Irradiated Frozen Aqueous Solutions as Revealed by ESR Spectroscopy." Book of Abstracts, 3rd Intern. Congr. Radiation Res. Cortina D'Ampezzo, p. 107, Tipografia Regionale, Roma, 1966.	
(1) RH 00193-03	MECHANISM OF THIOL PROTECTION AGAINST RADIATION	WALTER T. SMITH, JR., PH.D. DEPT. OF CHEMISTRY UNIVERSITY OF KENTUCKY LEXINGTON, KENTUCKY

Research Objectives: It is the purpose of this project to prepare a series of sulfinamides to be tested for antiradiation activity. Since sulfinamides readily oxidize thiols to disulfides, it is expected that the results of the testing will shed some light on the mechanism by which thiols protect against radiation damage.

Progress Report: A series of twenty-two substituted sulfinamides have been prepared for screening as radioprotective agents. Results of the screening will be reported when they become available.

The method of synthesis used the reaction of a Grignard reagent with an N-sulfinylamine.

$$RMgX + RNSO \longrightarrow RS\overset{\overset{O}{\|}}{N}HR'$$

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The compounds prepared are

1. N-p-methoxyphenyl benzenesulfinamide
2. N-p-methoxyphenyl m-methylbenzenesulfinamide
3. N-phenyl 2,4,6-trimethylbenzenesulfinamide
4. N-p-methoxyphenyl 2,4,6-trimethylbenzenesulfinamide
5. N-phenyl o-methoxybenzenesulfinamide
6. N-p-methoxyphenyl o-methoxybenzenesulfinamide
7. N-phenyl p-methoxybenzenesulfinamide
8. N-p-methoxyphenyl p-methoxybenzenesulfinamide
9. N-phenyl p-phenoxybenzenesulfinamide
10. N-phenyl o-chlorobenzenesulfinamide
11. N-phenyl p-chlorobenzenesulfinamide
12. N-p-methoxyphenyl p-chlorobenzenesulfinamide
13. N-phenyl 3,4-dichlorobenzenesulfinamide
14. N-phenyl p-phenylbenzenesulfinamide
15. N-p-methoxyphenyl p-phenylbenzenesulfinamide
16. N-phenyl p-dimethyl-aminobenzenesulfinamide
17. N-phenyl p-(4-morpholino)benzenesulfinamide
18. N-p-methoxyphenyl cyclohexanesulfinamide
19. N-phenyl 2-phenylethanesulfinamide
20. N-p-methoxyphenyl 2-phenylethanesulfinamide
21. N-phenyl 3-phenylpropanesulfinamide
22. N-p-methoxyphenyl 3-phenylpropanesulfinamide

As part of the characterization of the above compounds their infrared spectra were determined and show that the SO group of sulfinamides has characteristic absorption in the 1037-1055 cm^{-1} region. Data on the ir absorption of these compounds has not been previously available and will be published in Applied Spectroscopy during 1968.

Significance of Research: Two theories concerning the mode of action of thiols as protective agents against radiation suggest that the thiol acts either by forming a

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mixed disulfide or by reacting with free radicals formed by the radiation. The knowledge that sulfinamides oxidize thiols to disulfides enables us to test the above two theories by testing a variety of sulfinamides to see if they act as protective agents against radiation or as sensitizers to radiation. If thiols exert their protective influence via disulfide formation then sulfinamides should show some degree of protective action as a result of their reaction with natural thiol groups. However, if thiols protect by reaction with free radicals, sulfinamides will destroy any natural thiols present and act as sensitizers to radiation.

Results of this project could lead to the development of better protective agents against radiation either through the development of better thiol-type protective agents or by the development of sulfinamides as a new class of protective agent.

An incidental benefit of this project has been the acquisition of previously unavailable data on the infrared absorption spectra of sulfinamides.

Publications: None

RH 00222-05

STUDIES OF RADIOSTRONTIUM METABOLISM AND REMOVAL IN MAN

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Research Objectives: The aim of this investigation is to study the effect of certain compounds for the removal of radioisotopes, particularly of radioactive strontium from man after this radioisotope has entered the body. Another objective of this investigation is to find compounds which inhibit the entry of radioactive strontium into the body, that is, to prevent the absorption of the radioisotope from the intestine.

Progress Report: Several studies have been performed in this Research Unit in man on the removal of radioactive strontium and of other radioisotopes. These studies

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have shown that considerable amounts of radioactive strontium can be removed by the administration of calcium, magnesium or stable strontium by the intravenous route. The amounts of radioactivity removed depended on the time interval between the entry of the radioisotope into the body and the administration of these agents. A study of the comparative effectiveness of these three elements has shown that each of these compounds is effective in increasing substantially the excretion of radioactive strontium, thereby reducing the amount of radioactivity retained in the body. Intravenous calcium and intravenous magnesium were about equally effective while intravenous stable strontium was more effective than intravenous calcium or magnesium.

It has been reported that radioactive strontium can be removed via the saliva in experimental animals. This route of removal of radioactive strontium has then been investigated in man in this Research Unit. This study has shown that this route is a very inefficient way of the removal of radioactive strontium from the body and that the results obtained in animals were not applicable to man.

A simple method of determining the amount of radioactive strontium (strontium^{90}) which has entered the body with the food has been developed. This involves the determination of the excretion of calcium and of Sr^{90} in a single 24-hour collection of urine. The estimation of the amount of strontium^{90} present in the body is then calculated by the use of factors determined from the extensive information obtained in this laboratory with the short-lived strontium isotope Sr^{85}. This method is particularly useful in assessing the amount of strontium^{90} which accidentally entered the body.

Only very few substances are known to inhibit the absorption of radioactive strontium in animals and in man. Recently, the important observation has been made in this laboratory that the intestinal absorption of radioactive strontium can be decreased by an average of 87% by using a non-toxic medication (aluminum phosphate gel) which is widely used as an antacid for the treatment of patients with peptic ulcer. Further studies performed in this laboratory in experimental animals have shown that the

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administration of aluminum phosphate gel led to a marked decrease of the concentration of radioactive strontium in bone, by an average of 82%.

In regard to the detoxification of other radioisotopes, it has been shown that certain chemical agents which tightly combine with metals (chelating agents) were effective in removing radioactive zinc (Zu^{65}) as well as radioactive scandium (Sc^{46}) from the body.

A total of 12 publications have resulted from the investigations carried out with the support of grant RH 00222. Also, the results obtained have been presented before National and International Meetings.

Significance of Research: Strontium^{90} has been the subject of considerable study in regard to its concentration in bone, especially in the bone of children. Since this radioisotope resides in bone for very long periods of time and irradiates the bone marrow, methods for the removal of Sr^{90} became of importance. However, most studies on the removal of radioactive strontium have been carried out in experimental animals and only few studies have been carried out in man. Although the information obtained in animals is of great importance, recent investigations conducted by this group with the short-lived strontium isotope Sr^{85} have shown that the results obtained in animals are not always applicable to man. The studies performed directly in man under constant dietary conditions in this Research Unit have shown that several agents which are ineffective in animals are effective for the removal of radioactive strontium in man.

The significance of the investigations on the removal of radioisotopes from man lies in prevention of the long term effects which may be caused by the retention of radioisotopes in the body. Among the radioisotopes investigated is radioactive strontium which localizes in bone for many years and irradiates the bone marrow. This radiation effect may cause changes in the cells of the bone marrow which may lead to the development of leukemia. The agents used for the removal of radioactive strontium are non-

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toxic and are well tolerated if given in the amounts used in the studies described. The amounts of the detoxifying agents used in man have been well defined. The investigations have also shown that the effectiveness of these agents is greater the shorter the time interval between the entry of the radioisotope into the body and the administration of the detoxifying agents. The study of the effectiveness of several compounds for the removal of radioisotopes has established the comparative effectiveness of these agents.

The investigations concerned with the effect of aluminum phosphate gel on the prevention of absorption of radioactive strontium are of particular importance. These studies have shown that this non-toxic, well tolerated compound is indeed effective in inhibiting the absorption of radioactive strontium to a great extent in man. This compound is one of the most effective agents in preventing the entry of radioactive strontium from the intestine into the blood stream.

Publications:

Hart, H. and Spencer, H. "Rate of Initial Entry of Ca^{47} and Sr^{85} from the Intestine into the Vascular Space." Proceedings of the Society for Experimental Biology and Medicine, v126, 365-371, 1967.

Spencer, H., Lewin, I. and Samachson, J. "Inhibition of Radiostrontium Absorption in Man." International Journal of Applied Radiation and Isotopes, Vol. 18, pp. 779-782, 1967.

Spencer, H., Lewin, I. and Samachson, J. "Effect of Stable Strontium on Radiostrontium Excretion in Man." Radiation Research, Vol. 31, No. 4, August 1967.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00239-05	RADIATION PROTECTION AND RECOVERY IN GERMFREE ANIMALS	RAPHAEL WILSON, PH.D. DEPT. OF BIOLOGY UNIVERSITY OF NOTRE DAME NOTRE DAME, INDIANA

Research Objectives: Germfree animals afford a model system for assessing the effect of microorganisms on the response of mammals to whole-body ionizing radiation, the recovery phenomena, bone marrow therapy, and the action of protective agents. Using the approach of experimental pathology, an effort is being made to reproduce in germfree mice the pathology seen in conventional mice following whole-body irradiation in order to clarify the etiology of these changes with reference to microbial activity. The pathology of secondary disease in radiation chimeras is being investigated thoroughly, and with the use of antilymphocyte serum we are attempting to obviate even the mild symptoms seen in germfree chimeras and produce true tolerance to the bone marrow graft.

Progress Report:

A. Radiation Pathology

The LD_{50} X-ray dose for germfree Swiss Webster mice is about 50 R higher than that of the conventional controls. Their life span following a given dose of X-irradiation is longer (Wilson, 1963). In order to separate the damage to the hematopoietic system due directly to irradiation from that due to post-irradiation infection and an altered relationship to intestinal microorganisms, the peripheral blood changes in germfree and conventional Swiss Webster mice were compared for 24 days after whole-body X-irradiation (Wilson, Matsuzawa, and Connell, 1964). This study revealed less severe or less rapid depression of neutrophils and platelets in germfree mice exposed to midlethal and supralethal X-ray doses. It, therefore, appears that the presence of bacteria in irradiated animals contributes in part to damage to bone marrow as evidenced by a decrease in the peripheral count of cells originating in myeloid tissue, but does not alter the effect on lymphoid tissue.

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The erythrocyte and reticulocyte levels, however, of both germfree and conventional mice are significantly depressed at the midlethal and supralethal X-ray doses (Wilson, Matsuzawa, and Connell, 1964).

Because hemopoiesis is arrested in whole-body irradiation, a disturbance in iron metabolism could be expected as a sequel to irradiation. Secondary pathology resulting from iron imbalance may play an important role in radiation disease. Germfree and conventional CFW mice under control conditions present an almost identical serum iron pattern between the sexes and between the animal states. The monophasic diurnal variation of SBl levels in the two groups appear dependent on the daily activity cycle of the mice.

Following exposure to 850 R whole-body X-irradiation, different SBl response patterns were evoked in each group. The hyperferremia in X-ray treated germfree mice was sharply contrasted by the hypoferremia of X-ray treated conventional animals.

The possible involvement of bacterial endotoxin in provoking the hypoferremic state was seen in the marked decreases in SBl levels that it induces in both conventional and germfree animals, and in the typical conventional SBl pattern it causes when administered to X-irradiated germfree mice.

More explicit proof for the involvement of these vasoactive substances in radiation induced pathology came from data on irradiated gnotobiotic animals. Germfree mice monocontaminated with a gram-positive organism presented a post-irradiation SBl pattern similar to that of the germfree mice, whereas germfree animals monocontaminated with a gram-negative organism responded in a similar fashion to the conventional. Definitive evidence for the presence of vasoactive substances in the sera of irradiated conventional animals was obtained following the development of an endotoxin bioassay procedure. No similar material was found in the sera of irradiated germfree mice.

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Evidence that the serum iron decrease after X-irradiation was due directly to a loss of specific iron binding $beta_1$-globulin transferrin was presented, in which a 50% loss of protein was shown to occur in conventional mice 5 days after 850 R X-irradiation.

The gross decrease in total serum protein and albumin levels that accompanied the administration of endotoxin gave support to the hypothesis that accompanying the alteration in the vascular permeability a leakage of serum had occurred.

The results of the study are consistent with the following proposed mechanism of action for the induction of hypoferremia in X-irradiated conventional animals: 1) X-irradiation produces conditions that favor the absorption of endotoxins; 2) bacterial products that are vasoactive substances cause changes in the permeability of the vessel membranes, either directly or indirectly through other agents; 3) this increased permeability allows serum proteins to leak out into surrounding tissue spaces, resulting in a decrease in the transferrin level and an ultimate hypoferremia.

An extensive hematological study of the peripheral blood and bone marrow and a histopathological study of germfree and conventional C3H, CFW and BALB/c mice exposed to X-irradiation over a dose range of 500-8000 R is in progress. All the tissues have been collected, but the histological study has not been completed to date.

B. Radiation Protection

With the intraperitoneal administration of 10 ug of Boivin prepared E. coli endotoxin 24 hours before whole-body X-irradiation, the LD_{50} X-ray dose is raised approximately 100 R for both groups of mice; for germfree mice it is 942 R and for conventional mice 829 R. Since the X-ray dose reduction factors are nearly identical, it seems that the reactions to small doses of endotoxin which confer radiation protection in mice are independent of any reactions to endotoxin released by endogenous bacteria.

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The absence of a bacterial intestinal microflora did not alter responses to endotoxin associated with radiation protection in conventional mice. In germfree mice, as in conventional, endotoxin provided in multiple inoculations one week prior to or immediately before or after X-irradiation did not induce protection. The best radioprotection by endotoxin was provided by one inoculation, 24 hours before X-irradiation.

C. Radiation Treatment

Bone marrow transplants have been the most successful treatment for the lethal hematopoietic damage in conventional mice, although some animals so treated still die of infection. This method of treatment has been used in germfree mice to assess the effect of the animal's microbial associates on the ability of bone marrow transplants to prevent death within a 30-day observation period.

Conventional C3H mice which received isologous bone marrow had an LD_{50} of 860 R, whereas it was 750 R for those which received homologous (CFW) marrow. Treatment of germfree C3H mice with isologous marrow resulted in an $LD_{50(30)}$ of 1,350 R, while it was 1,325 R in those receiving homologous treatment, and increase of 500 to 600 R over that of conventional mice.

The LD_{50} of conventional CFW mice treated with homologous marrow cells was 850 R, while that of germfree animals which received homologous (C3H) marrow was 1,250 R, a difference of 400 R. For isologous transplants the difference between the LD_{50} of conventional and that of germfree animals was 700 R (the LD_{50} being 900 R for conventional mice and 1,650 R for germfree mice). In all groups the mean survival times of the animals that died during the 30 days observation period did not differ significantly from those of untreated controls.

Significance of Research: We have perhaps been a bit naive to date regarding the role played by microorganisms in radiation pathology. While there has been little doubt that bacteria invade the irradiated animal, it has not been clear what pathology is the direct consequence of infection. It is an oversimplification to say that an answer can be found by irradiated germfree mice and comparing the pathology

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of these mice with that of conventional mice and attributing the difference to infection. The germfree mouse is not just a mouse without a bacterial flora; it is an animal in which key organs of significance in radiation disease are histologically and physiologically different, in degree if not in kind, and will respond differently to radiation. In other words, we now feel that a major contribution of germfree studies will be delineating the effect of certain bacterial substances in tissues, organs, and systems and noting how these affect the response to radiation and to treatment. When these effects are known, a program for control of these substances can be initiated and a practical schedule for radiation treatment can be developed.

Publications: No additional publications

(1) RH 00245-04 DEHYDROGENASE KINETICS "UNDER THE BEAM"

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Research Objectives: Dehydrogenases from various tissues in several animals are being studied during X-irradiation to determine whether the previously observed immediate and transient decrease in reaction velocity of NADH oxidase during irradiation is a general property of NAD-linked dehydrogenases.

Progress Report: A reversible decrease in the reaction rate of an NADH-FMN dehydrogenase obtained from luminous bacteria has been observed during X-irradiation (Burns, Radiation Research, 15:178 (1965)). In an effort to determine whether this behavior was peculiar to this enzyme system or a more general property of NAD-linked dehydrogenases, the following enzyme systems were examined during irradiation: rabbit-muscle glyceraldehyde-3-phosphate dehydrogenase, horse-liver alcohol dehydrogenase, yeast alcohol dehydrogenase, pig-heart malate dehydrogenase, and beef-heart lactate dehydrogenase. The reactions were followed by measuring the absorbance of NADH at 340 mu. A dose rate of 60 rads/sec/ of 50-kVp X-rays was used. In each reacting enzyme system the reaction rate during irradiation, when corrected for non-enzymatic

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radiation induced changes, was less than that of the control at the same time in the overall reaction. The change in rate started promptly with exposure to X-rays and persisted only during exposure. Rates during irradiation varied for individual enzyme reactions from approximately 90% of control to 67% with glyceraldehyde-3-phosphate dehydrogenase.

The cytochrome c reductase and yeast lactate dehydrogenase reactions were also studied. During irradiation at 60 rads/sec. their reaction rates are approximately 58% and 9% of controls, respectively. These results add further support to the involvement of temporary interference with enzyme kinetics in the production of latent radiation injury.

In order to make these measurements, an apparatus was constructed to permit continuous spectrophotometric observation during exposure of a sample to X-radiation. The sample compartment of a spectrophotometer was modified to encompass an X-ray tube and a specially designed sample cell. Provisions were made to control the temperature and gaseous environment of the sample and to carefully select the layer of solution being observed.

Significance of Research: This work demonstrates that enzymes and their reaction rates can be immediately and transiently altered during irradiation. This alteration occurs at doses considerably lower than those required to produce permanent enzymatic changes as determined by post-irradiation assay. Because the enzymes examined are involved in energy metabolism, the possibility is strengthened that cell injury may arise as much from temporary disruption of the balance of cell dynamics as from the production of irreversible chemical damage.

Publications:

Riley, R. C. and Nevill, A. D. "Measurement of Enzyme Reactions During X-Irradiation." Radiation Research, Vol. 31, No. 3, July 1967.

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RH 00245 (Continued)	Riley, R. C. "Spectrophotometry of Biochemical Solutions During X-Irradiation." Paper presented at the Third International Congress of Radiation Research, Cortina d'Ampezzo, Italy, July 1, 1966. Riley, R. C. and Burns, C. H. "Changes in the Oxidation-Reduction State of Cytochrome c in Aqueous Solution During X-Irradiation." Radiation Research, Vol. 27, No. 3, March 1966.	
(1) RH 00246-03	EFFECTS OF RADIATION ON THE MIGRATION OF SALMONIDS	FRANK P. CONTE, PH.D. DEPT. OF ZOOLOGY OREGON STATE UNIVERSITY CORVALLIS, OREGON

Research Objectives: The purpose of the study is to understand the molecular and cellular mechanisms responsible for the control of salt and water movement across biological membranes.

Progress Report: Current work in this laboratory has explained the cellular dynamics that occur in the biological membranes of the gill structures of salmon. Recent autoradiographic experiments have revealed that ^{3}H-thymidine, a DNA precursor, is rapidly incorporated into the genetic material of certain types of cells located within the gill filaments. Time and motion studies of these cells have shown that if the gills are exposed to X-rays, these cells and their development are destroyed along with the ability of the animal to live in sea water. These experimental findings suggest that the control of the permeability of the gill, which regulates the amount of salt and water passing into the animal, is dependent upon the rate of development of these specialized cells. Electron microscopic investigation of the specialized cells show that they possess a unique ultrastructure. They have a large population of mitochondria and a great quantity of smooth intracellular membranes. The biosynthesis of the intracellular membranes is thought to be responsible for the mechanism of transport of the ions.

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Significance of Research: Radiation is injurious to the gill system of marine fishes. It has been experimentally shown that disturbance of the cellular dynamics of the gill structure can lead to the death of animals which utilize this system as the major organ for regulating mineral and water balance. This model cell system may be ideally suited for studying the pathological changes which occur in the renal tubule system of man following radiation exposure. In addition, this basic information is needed for ecological studies of the estuary and marine environment which deal with radionuclides so that a better judgment of the radiosensitivity of marine organisms can be achieved.

Publications:

Conte, F. P. and Morita, T. "Immunochemical Study of Cell Differentiation in Gill Epithelium of Euryhaline Oncorhynchus (Walbaum)." J. Comp. Biochem. Physiol. 24:445, 1968.

Conte, F. P. and Lin, D. H. "Kinetics of Cellular Morphogenesis in Gill Epithelium During Sea Water Adaptation of Oncorhynchus (Walbaum)." J. Comp. Biochem. Physiol., Vol. 23, pp. 945-957, 1967.

RH 00268-05 LOCAL CHEMICAL RADIATION PROTECTION IN MICE

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Research Objectives: The principle purpose of this project is to determine the degree and character of radiation protection afforded by different chemical agents applied directly to the mouse tongue. The chemical agents being used are mercapto-ethylamine, MEA, aminoethylisothuiromiun, AET, and para-aminopropriophenone, PAPP. The assessment of protectior is determined by the ability of these chemicals to alter the mortality of oral radiation death and their ability to prevent or alter the sequence of histologic changes in the irradiated tongue which precede death. It is expected that the results of these studies would provide information which

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would be necessary for an evaluation of the feasibility of using radiation protection chemicals to protect normal tissues during radiation therapy for cancer.

Progress Report: Prior studies have indicated that oral radiation death appears to be caused by dehydration and starvation which, in turn, are due to lack of ingestion because of a consistent, radiation induced, ulceration of the posterior area of the tongue. Death occurs approximately nine days after irradiation.

The injection of each of the radiation protection chemicals, MEA, AET, and PAPP produced appreciable protection against the mortality of oral radiation death, raising the dose of radiation necessary to kill 50% of the mice to about 300 roentgens above the dose of radiation required to kill 50% of the nonprotected mice. The chemical MEA appeared to give more protection than AET or PAPP. However, when the amount of PAPP was increased, there was increased protection; at near toxic doses the degree of protection surpassed that afforded by MEA. Previously it was believed that the mechanism through which PAPP gave radiation protection was by decreasing the oxygen content of tissues. Decreases of oxygen are known to increase tissue resistance to radiation and conversely increases in oxygen content generally increase tissue sensitivity to irradiation. PAPP alters the hemoglobin in such a way that it cannot carry oxygen and the tissues quickly suffer oxygen deprivation. The present studies demonstrated that while increased doses of PAPP did increase the radiation protection, the percentage of hemoglobin change did not increase above the basic level achieved with the smallest dose of PAPP. Further, irradiation of mice while under hyperbaric oxygen conditions did not destroy the relative protection produced by PAPP. Nor did the methylene blue reconversion of the altered hemoglobin to oxygen carrying hemoglobin before irradiation change the protective ability of PAPP. Hence it was concluded that the radiation protection mechanism of PAPP could be independent of tissue oxygen levels.

Microscopic examination of the tongues from mice receiving injected chemical protection and irradiation as contrasted to those mice receiving only irradiation indicated that the kind of radiation induced changes were about the same in both groups

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of mice during the first seven days after irradiation except that the rate of change was less in the chemically protected animals. Following the first week after irradiation, the protected mice had sudden recovery of the radiation damage to the tongue tissue while in the nonprotected mice the radiation induced changes continued to progress leading to the lethal mechanism of oral radiation death.

The local application of MEA and AET to the mouse tongue produced degrees of protection better than those achieved by the injected use of the same chemicals. Repeated trials with PAPP used locally on the tongue failed to demonstrate any degree of protection against oral radiation death. The microscopic examination of the tongue tissue during the days following irradiation revealed minimal changes in animals protected with MEA and AET with complete restoration to normalcy nine days after irradiation, the time when the nonprotected mice were suffering the most severe radiation induced injury. Some protected mice did have the usual post-irradiation progression of injury and there were deaths of protected animals. The tissue injury however was confined to the back area of the tongue which is the most critical area related to mortality. The front portion of the tongue had minimal changes with good recovery after one week although the front portion of the tongue in nonprotected mice always had severe radiation changes. This was interpreted as a failure to consistently include the back area of the tongue in the local application. More deliberate attempts to include the back area of the tongue in anesthetized mice resulted in a very high incidence of mortality due to choking or pneumonia. However, this experimental occurrence provided the opportunity to observe that the distinction between protected and nonprotected areas of the tongue was very abrupt; there was no "spill over" of protective effect. This is considered to be a definite asset when related to the possibility of controling radiation effects within discreet tissue areas. In addition, it is probable that the mortality incidence of oral radiation death does not fully reflect the possible degree of local chemical protection which could be achieved since many deaths were probably due to the experimental difficulties in consistently protecting the most critical area.

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The basic radiation induced defect appears to be inhibition in the replacement of the epithelial cells of the tongue. Normally new cells are produced at the base of the tissue, these mature and finally are desquamated at the surface. A halt in the production of new cells will lead to a loss of the tongue epithelium in about one week. Thus the fundamental locus of action by chemical protection seems to be the protection of the cell replacement mechanisms of the epithelium. The production of new cells depends upon DNA synthesis and cell division. Hence an evaluation of the production of new cells after irradiation in both chemically protected and non-protected mice should be a more direct and accurate measure of the possible degrees of local chemical protection than the mortality incidence of oral radiation death. Presently studies are in progress which are directed at measuring comparative DNA synthesis rates and cell division rates in the mouse tongue after irradiation of protected and nonprotected mice.

The data which has already been obtained from these latter studies indicates that although the incidence of DNA synthesis is not drastically reduced during the days following irradiation and mitotic figure incidence is depressed only slightly more, the tongue does suffer atrophy of the epithelium until seven days after irradiation when there is a sudden replacement of lost epithelial layers. This suggests that there may be a coaction between the cells which produce replacement cells. Hence even though cell division is taking place prior to the seventh post-irradiation day a meaningful contribution to the whole tissue cannot be made until the seventh day. To investigate this phenomenon, a technic has been developed which will allow for the microscopic observation of all the generating cells at one time within their apatial relationship. Presently only one row of these cells can be seen at one time in the cut sections. This technic utilizes a chemical which causes blistering in the epithelium above the generative cell layer, thus removing the cells above and an enzyme digestion of the tissues below the generative cell layer. This has been accomplished and it remains to develop this technic into a usable laboratory procedure giving consistent results.

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Significance of Research: While this specific study is not directly concerned with human use, the ultimate goal of this study is to increase the differential effect of radiation therapy between cancerous and contiguous noncancerous tissues in humans. This would be accomplished by applying radiation protection chemicals to noncancerous tissue surfaces or body cavities which would be desirable for two reasons. Primarily the integrity of normal tissues would be maintained. Secondarily, since the healing of the destroyed tumor areas proceeds from surrounding normal tissues, post-treatment healing should be enhanced.

The results of radiation therapy in the oral areas are particularly unfortunate. Salivary processes are often halted permanently. The continuing dry mouth is of extreme discomfort to patients. Salivary glands could be protected by retrograde application through the duct system. Further the oral surface tissues and jaw bones have lost their usual ability to resist infection and repair injury. Infections in irradiated jaw bone are sometimes more difficult to treat than the original cancer and lead to serious debilitation and cosmetic difficulties for patients. Chemical radiation protection could also effectively be used in other areas of the body.

The results of these studies about local chemical protection in mice have given information which indicates that human use of local chemical protection in radiation therapy may be clinically feasible.

Publications:

Goepp, R. A. "Topical Chemical Protection Against Oral Radiation Death in Mice." Radiation Research, Vol. 34, No. 1, April 1968.

Goepp, R. A. and Fitch, F. W. "Effects of Radioprotective Chemicals on the Histopathologic Changes of Oral Radiation Death in Mice." Journal of Dental Research, Vol. 46, No. 3, May-June 1967.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00279-05	DISPLACEMENT REACTIONS OF ANTI-RADIATION DRUGS	ROBERT E. DAVIS, PH.D. DEPT. OF CHEMISTRY PURDUE RESEARCH FOUNDATION LAFAYETTE, INDIANA

Research Objectives: The objective of this research is to study the chemistry of sulfur containing irradiation drugs with specific reference to the ease of their ability to function as nucleophiles in S_N2 reactions. These studies of the physical organic chemistry of the irradiation drugs should aid in the interpretation and elucidation of radioprotective mechanisms.

Progress Report: The rates and mechanisms of reactions of compounds as MEA (mercapto-ethylamine) with a variety of substrates as methyl iodide, ethyl tosylate and β-propiolactone have been studied. The Oxibase Scale has been applied with success to these displacement reactions of numerous nucleophiles including the anti-radiation drug, MEA, on sulfur-sulfur, oxygen-oxygen, and carbon-X bonds. It is concluded that the ease of oxidation of the nucleophile is of prime importance to obtain fast reaction rates and favorable equilibria. Of secondary importance is the base character of the nucleophile. The quantitative scale relating these two terms has been called the Oxibase Scale.

Significance of Research: The thesis of this proposal is that activity of anti-radiation drugs can be correlated to the oxidation potential, basicity and the degree of participation of the drug. Understanding of these mechanisms allows better predictions of structure, reactivity and synthesis of more useful compounds. We hope to make some new contributions to anti-radiation drug design.

Publications: No additional publications

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RH 00280-05	EFFECTS OF RADIATION AND DRUGS ON HAIR AND HAIR GROWTH	FREDERICK D. MALKINSON, D.M.D. DEPT. OF MEDICINE/DERMATOLOGY UNIVERSITY OF CHICAGO CHICAGO, ILLINOIS

Research Objectives: Rapidly dividing hair cells are used as a model system to study the biological effects of radiation alone, or combined with drugs, duration of cellular injury, and character of recovery. Results of dose fractionation, comparisons of different radiation modalities, and radiation effects on cell division are currently under investigation. Electron microscope studies of ultrastructural changes characterizing radiation injury are in progress.

Progress Report: Although the cosmetic role of hair far outweighs its physiological significance, hair-producing cells are among the most rapidly proliferating in the body. Since hair formation depends on continuous large-scale cell production, even minor injury to these cells results in hair defects which can easily be detected and measured. Ionizing radiation or cell-damaging drugs produce thinning and irregularities of the hair shaft. Microscopic studies reveal that the severity of injury and the number of hairs affected increase with increasing dosages. Also, the slowed growth rates of damaged hairs result in shortened hair lengths. Reduced growth rates and subsequent recovery can be accurately measured in laboratory animals by repeated injections of isotope-labeled amino acids, which are incorporated into hair proteins. Removal of single large hairs (vibrissae) for placement on unexposed photographic film yields autoradiographic images which contain easily identifiable "spots" corresponding to amino acid injection times. Decreased size of these "spots" and diminished distances between them, compared to normal hairs, reflect the magnitude and duration of hair matrix damage. Large plucked samples of body hair from animals given isotope-containing amino acids can be directly assayed for radioactivity by sensitive detectors. Agents injurious to hair cells depress such amino acid uptake, and the magnitude of this depression is again dose-dependent.

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All of these techniques, developed or refined in our laboratories, have been adapted to the study of hair and hair cells as a representative model for tissue injury and recovery produced after exposure to radiation alone, pharmacologic agents, or combined administration of radiation and drugs. Use of the microscopic technique to detect hair thinning revealed that two chemotherapeutic agents - colchicine and actinomycin D -, utilized for tumor therapy, each greatly enhanced radiation damage when given in very small dosages to mice four or 16 hours prior to radiation. These synergistic effects were not found when the sequence of drug-radiation administration was reversed. The usefulness of hair matrix populations as a model cell system was confirmed by findings of similar drug-radiation effects in some animal tumors and, subsequently, in certain human malignancies. The clinical usefulness of repeated drug-radiation treatments has since been evaluated in over 150 patients with inoperable malignancies. Therapy of gastro-intestinal tract tumors and pulmonary cancers in particular has resulted in significantly increased comfort and longevity for many patients, in some of whom complete eradication of the primary malignancy has been obtained. The current limitations of this treatment regimen reflect our inability to detect early foci of tumor spread for all tumors, as well as the occurrence of some malignancies which are completely resistant to therapy. The cyclic 4 and 16 hour character of colchicine-radiation-induced cell damage may reflect profound effects on the cell's reproductive cycle, since cells are known to be more radiosensitive in some phases of the generation cycle than in others. This problem will be investigated at the tissue microscopic level with biopsy material and commonly used autoradiographic techniques to study generation cycle effects in cells containing radioactive (tritium-labeled) DNA.

Hairs do not grow continuously, but undergo periods of rest (telogen). In a telogen hair no cell division is detectable. After a short interval, growth of a new hair begins spontaneously with active cell division (anagen phase), and the old hair is pushed out of the follicle. This entire cycle is repeated at similar intervals (six weeks for mice, three years for the human scalp) throughout a lifetime. Studies of large hair samples in mice given radioactive, tritium-labeled amino acid (serine) revealed markedly impaired serine uptake in anagen hairs four days after irradiation.

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If telogen hairs were irradiated at the same dose level and then plucked to induce anagen, serine uptake in these new hairs four days later was reduced only half as much as for the irradiated anagen hairs. This confirmed older observations that non-dividing cells are significantly more resistant to X-ray damage than dividing cells. We then found that whether irradiated telogen hairs were allowed to remain in the follicle for as little as one day or for as long as twelve days, plucking to induce anagen was followed by exactly the same degree of impaired serine uptake for both time intervals. These studies indicate that X-ray damage can be "stored" in resting hair cells for long periods, and that even partial recovery cannot be demonstrated. By contrast, irradiation of anagen hairs is followed by a return to normal serine uptake twelve days later, indicating complete recovery of protein synthesis in proliferating matrix cell populations. Injured telogen cells, induced to divide by hair plucking, immediately after injury, show the same accelerated recovery rate as matrix cells damaged in anagen. Dividing cells, then, are more susceptible to radiation damage than non-proliferating cells, but conversely these actively metabolizing cells also recover from radiation injury far more rapidly and effectively. Since different cell systems throughout the body have widely varying rates of cell division, implications of these observations for tumor radiotherapy on the one hand and environmental radiation hazards on the other must be investigated further.

Hair growth rates reflected in overall hair length measurements are a highly sensitive indicator of radiation damage. Daily irradiation of mice with small doses of gamma rays or neutrons for periods of one to six months produced up to 25% reductions in hair lengths for the longest exposure periods. For neutron irradiation, significant hair length changes were observed at dose rates of only 3 rads daily within three and one-half weeks after studies were begun. No other radiation-induced physical changes could be demonstrated in these animals at such short treatment intervals. These studies provide a method for gross biological detection of radiation in doses smaller, perhaps, than can be detected by any other technique yet demonstrated. The possible usefulness of this method in measuring small extraneous doses of radiation at the source for measurements of radiation effects in space exploration, or even for retrospective dosage estimates in radiation accidents are obvious.

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A 25-30% retardation of hair growth produced by large single doses of radiation (1,600-2,000 rads) persists indefinitely in mice throughout subsequent hair cycles. These findings indicate that severe hair matrix cell damage is transmissible from one hair "generation" to all subsequent hair generations without apparent recovery. This observation provides unique opportunities for long-term morphologic and biochemical studies of persistent radiation damage at the cellular level.

Significance of Research: Increased knowledge of the effects of radiation on proliferation, injury, and recovery processes in hair matrix cells may have broader significance for our better understanding of and protection against radiation effects in other tissues and organs. Further studies of combined radiation-drug administration on cell division and injury may increase our knowledge of radiation synergism and radiation protection. These findings could also be important to a better understanding of enhanced therapeutic results following X-ray-drug treatment of human malignancies. Methods developed for measurement of radiation effects in hair provide suitable techniques for detection and dose estimation following even small exposures to radiation. Studies in progress on the ultrastructural alterations of irradiated hair matrix cells may have broad significance for radiobiological effects in other cell systems.

Publications:

Malkinson, F. D. and Griem, M. L. "Radiation Injury and Recovery in Anagen and Telogen Rodent Hairs." Radiation Research 33, 554-562, 1968.

Griem, M. L. and Malkinson, F. D. "Some Studies on the Effects of Radiation and Radiation Modifiers on Growing Hair." Radiation Research 30:43, 1967.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00293-04	α-ALKYLOXIMINOMERCAPTANS FOR PROTECTION IN RADIOTHERAPY	DOMINICK A. COVIELLO, PH.D. DEPT. OF CHEMISTRY UNIVERSITY OF ILLINOIS CHICAGO, ILLINOIS

Research Objectives: The main objective of this research is to prepare synthetic chemicals for use as protective agents against ionizing radiation, and to obtain information from model compounds to aid in the design of more potent protective agents. The ultimate objective is to make available compounds which are effective in small doses whether administered before, during or after lethal or sublethal doses of ionizing radiation.

Progress Report: In the proposed scheme for the synthesis of the title compounds, it is necessary to proceed through several steps. In each step, a product is obtained and purified. Scrupulous purification is necessary in order to aid in characterization.

With the intermediate compounds and end products, it is felt that they should also be screened for biological activity other than that for which they are prepared. The reason for this is to guard against the possibility of overlooking unusual or unexpected effects.

Since small amounts are necessary for testing, and since the test systems are available at the Walter Reed Army Institute of Research where the testing is done, all of the compounds sent there are screened for antiradiation, antimalarial, and antishistosomiasis activity.

Of those compounds for which test data are available, the expected as well as some unexpected activities have been found. Of the cyanide derivatives tested, one offered slight protection to mice that were pretreated with the compound before exposure to X-rays. The interesting thing was that the toxicity of the active compound was lower by a factor greater than 30 when compared with the related

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compounds that were not at all protective. Several explanations can be offered which could clarify the observations. This, however, can be proved only with more extensive biological work, which hopefully will soon be forthcoming.

As a possibility, one could assume that all are acting by the same mechanism, namely by the release of hydrogen cyanide which is known to be protective. In the case of inactive compounds, there may be a critical dose below which the postulated slow release of hydrogen cyanide results in concentrations too low to be effective, and above which hydrogen cyanide release is so fast that it exceeds the amount the animal can tolerate.

Probably the most striking aspect of the test results is the antimalarial activity demonstrated by a new class of compounds never before prepared or so tested. While the activity was moderate in most cases, some of the compounds were able to increase the survival time of mice infected with malaria up to 3.3 days longer than controls. The compounds in question are α-alkyloximinoacyl thiols.

To explain the mechanism of the antimalarial activity, it is necessary to do so a priori since no related compounds have been reported to have such effects. One could presume that perhaps there is interference with a vital enzyme system which could conceivably be responsible for conversion of an oxidized system to one that is reduced. Such reactions are numerous in living cells, and the oxime grouping contained in the molecule being amenable to reduction could assume the role of a normal substrate.

In the preparation of some of the compounds described, we have been able to improve upon established methods. The improvements have resulted in better yields of products of higher purity in less time. This is particularly so in the preparation of thiol ester intermediates.

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The halogenation of O-alkyloximes to yield α-bromo-O-alkyloximes is an application of a well-known reaction which had not been explored previously. It is interesting that in some cases the bromides do not react in the usual way to yield the expected title compounds. This is rather striking in that oximes, which are ketone derivatives, do not usually behave as ketones as our observations appear to indicate. Said observations may be useful in unravelling some of the mysteries of the often overlooked oximes - especially the O-alkyl derivatives.

Significance of Research: The fact that biologically active compounds have been obtained is significant, especially since a new class of compounds are involved. Finding chemicals with biological activity is always a happy situation. It is especially good when the compounds are of a type not previously explored. Said compounds offer a new point of departure for attacking the problem of obtaining new and potent drugs. They also offer a new point of attack for the elucidation of drug action since the accumulation of data relative to the mechanism of drug action offer better opportunities for correlations. With good correlations, a more rational approach to drug design is possible.

In short, the present investigation has furnished new leads for agents which can serve as antimalarial and radioprotective drugs.

Publications: No additional publications

(1) RH 00297-04 RADIOPROTECTIVE BASIC THIOLS

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Research Objectives: The primary purpose of this project was to find more effective radiation-protective agents than are now available, e.g., agents having greater protective ability or lower toxicity. The agents were designed to react preferentially in the animal with metalloenzymes, particularly those involved in cellular oxidations.

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Biochemical studies were carried out to show whether the agents do combine with enzymes of this type.

Progress Report: Acylthio derivatives of 2-mercaptoethylamine (MEA) and 2-mercaptoethylguanidine (MEG) having reactive sulfur-containing functional groups are the most effective radiation-protective agents known. With the assumption that radiation-protective compounds are involved in complexation with or binding to enzymes or other vital cellular molecules, other compounds containing both basic and reactive sulfur-containing functions were synthesized. Replacement of the basic function with the cyano group was also made, since the cyano group is also a complexing function, particularly with metal ions.

Compounds of these types which were synthesized include the following: amino dithio esters and amino iminothio esters related to α- and β-amino acids, aromatic and metal-containing derivatives of 3,3-dimercaptoacrylonitrile, cyanoalkylthiosulfates, dithioacetic acid pyridinium betaines and esters, dithiocarbamates of strongly basic heterocycles, dithiocarbamates and trithiocarbonates of substituted thiophenes and furans, trithiocarbonates of long-chain alkyl derivatives of MEG, the thiolsulfinate and N-cyano derivatives of MEG, the dithiocarbamate trithiocarbonate of cysteine, and various condensation products of carbon disulfide with aromatic amines, aromatic amino halides, sulfonamides, and a nitrile oxide. Radiationprotective activity was found in most of these series of compounds, which represent new types of structures for this activity. In particular, the protective activity of the dimercaptoacrylonitriles has given for the first time a series of non-basic thiols with anti-radiation properties. It is probable that these compounds protect by means of reaction with radiation-produced free radicals rather than by enzyme complexation. Also, the activity of the dithioacetic acid pyridinium betaines and esters has provided further evidence for the importance of charged or zwitterionic structures in radiation protection.

The most effective radiation protector found in this project was the dithiocarbamate trithiocarbonate of cysteine, which was equal in potency to MEG but 2-3 times less

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toxic. A metabolic study of this compound in mice showed the dithiocarbamate function to be stable in vivo, and the trithiocarbonate function to be unstable, which led to the conclusion that the dithiocarbamate was the protective species.

Enzyme inhibition studies were carried out with a series of radioprotectors and the metalloenzymes catalase and beef heart lactic dehydrogenase. A rough correlation between enzyme inhibition and radiation-protective activity was observed. Using a non-metalloenzyme, rabbit muscle lactic dehydrogenase, scarcely any inhibition was found for these agents, which points to the importance of binding to metalloenzymes involved in cellular oxidations for radiation protection.

Significance of Research: A radiation-protective agent having equal potency to MEG, one of the most potent radiation-protectors known, and having about one-third its toxicity was found. Several new types of radiation-protective compounds were discovered, which provides new bases for further synthetic attempts. The importance of binding to oxidative metalloenzymes by basic mercaptans and their S-acyl derivatives for radiation protection has been shown. A series of non-basic thiols was found to be radiation-protective, probably by reaction with radiation-produced free radicals. The latter compounds are closely related to agents presently employed for sutureless surgery and are well tolerated by the body.

Publications: No additional publications.

(1) RH 00305-04 INACTIVATION OF ENZYMES BY IONIZING RADIATION

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Research Objectives: The objectives of the present project are to study the inactivation of enzymes by X- and γ -radiation, with special reference to conditions like those occurring in biological systems (i.e., concentrated solutions and suspensions). For some enzymes, whose structure and mode of action is sufficiently

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well-known, we seek to relate the inactivation to the occurrence of definite chemical changes and to correlate the radiation response with other properties of the molecules. Finally, we are looking for ways to modify - either reduce or enhance - the effects of radiation by means of appropriate chemical treatments or changes in condition.

Progress Report: In Part I of the report are listed the principal projects that have been completed or are now in progress, with bibliographic references to papers that have been published. In Part II, a more coherent, logical discussion is given of the salient results and conclusions, together with some pertinent results from other sources.

Part I

(1) Mercaptan-Disulfide Interchange and Radioprotection (Progr. Biochem. Pharmacol. 1, 142-51 (1965)). Values of the equilibrium constants for several mercaptan-disulfide interchange reactions are reviewed. Since in most cases the values are near 1 and there is no clear correlation to radioprotective ability it is suggested that protection by mercaptans and disulfides probably is due mainly to radical scavenging and repair.

(2) Inactivation Yields in the X-Ray Inactivation of Enzymes. Representative Values and Some Problems (Abstract, Third Internat. Congr. Radiat. Res., June 1966). Values for the inactivation of several enzymes are reviewed. It is suggested that a comparison be made on the basis of weight inactivated per unit dose, inasmuch as molar inactivation yields tend to give low values for larger enzymes.

(3) Equilibria in Thiol-Disulfide Interchanges. Part V. Reaction of Some Aminoethanethiols with 4,4'-Dithiobis(benzenesulfonic Acid) (J. Chem. Soc., London B, 1967, 729-36). Equilibrium constants for the interchange of the title disulfide with 2-aminoethanethiol and 8 derivatives show no obvious correlation with radioprotective ability. This is in accord with (1).

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(4) Inactivation of Alcohol Dehydrogenase (YADH) by X-Rays and the Dose-Modifying Effect of Some Added Substances (Radiat. Res. 32, 671-84 (1967)). The inactivation yield is 0.14 molecules/100 ev, constant in the range 0.1-1 mg/ml. Added mercaptans and disulfides, also some other substances, have small dose-modifying effects at 50:1 molar ratio.

(5) Inactivation of Aldolase in Solution by X-Rays (Experientia, 24:440, 1968). The yield is 0.10. The protection-sensitization pattern is quite different from that of YADH.

(6) Effects of Radiation on Lysozyme. In dilute solutions, the inactivation-dose relation is near linear, the yield 0.18. p-Aminobenzoic acid (PABA) and phenylalanine protect the enzyme in proportion to their rate of reaction with OH; added nitrate has little effect. Attempts are now being made to characterize the products by gel electrophoresis and amino-acid analysis.

(7) Inactivation of Other Enzymes. For urease, the inactivation yield is 0.015; this seems a very low value but the weight inactivated per unit dose is 7×10^{-11} g/erg, near the middle of corresponding values for other enzymes (cf. (2)). For α-amylase, the inactivation-dose curve is definitely intermediate between linear and exponential. A procedure is proposed for calculating the yield in such cases; the result is 0.08 (cf. also (9)). Urease is well protected by PABA, little affected by nitrate.

(8) Inactivation of YADH in Concentrated Suspensions and Solution. Lyophilized enzyme was mixed with 0.1 to 10 parts of water and irradiated. The apparent G value, calculated from the total dose administered, decreases monotonically from about 3 to 0.3 . D_{37} has a maximum at about 45% water. It is suggested that the classical concepts of "direct" and "indirect" action are not applicable, a more appropriate interpretation is being developed.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00305 (Continued)	(9) Inactivation-Dose Relationships in Irradiated Enzyme Solutions (I presented at the 6th Annual Meeting, European Society of Radiation Biology, June 1968). Inactivation-dose relationships have in many cases been interpreted in terms of an "average" rate constant that is (nearly) the same for native and inactivated enzyme and that remains unchanged throughout the irradiation. There are reasons for regarding this model as inadequate. A treatment based on more realistic assumptions is being developed, that can be applied as well to non-exponential cases (such as (6) and (7)).	

Part II.
(The numbers in parentheses refer to the investigations listed in Part I.)

The results of many workers, notably those of Pollard and co-workers, indicate that the sensitivity of enzymes in the solid, dry state increases with increasing molecular weight (M). The relation $M = 7 \times 10^{11}/D_{37}$ describes the behavior of many enzymes approximately, within a factor of 2 to 3; i.e., 100-300 ev suffices to inactivate one enzyme molecule. This can be understood on the basis that the transfer of energy occurs in "packets" of about 100 ev and that the deposition of one packet in a molecule inactivates it, regardless of its size (to the aforementioned degree of approximation).

The behavior in dilute solution, 0.1-1 mg/ml, is completely different. In general, the larger the enzyme the lower the yield (2), but there is a larger variation in the ratios between the mass and the inactivation dose. Most yields are in the range 0.05-0.5 molecules/100 ev (4, 5, 6, 7).

The results can be rationalized in the following way. Radiation is absorbed first by the water, which is thereby decomposed: 2-3 $OH^{\cdot}$ radicals and hydrated electrons (e_{aq}) are formed per 100 ev. In a typical enzyme, these radicals can react with many amino-acid residues and from the values of the inactivation yield one must deduce that only a few of the reactions are effective in causing inactivation. The remainder presumably involve already inactivated enzyme or sites that can be altered without loss of activity. In general, the larger the enzyme the more sites it will

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contain, hence the lower the yield (2). The occurrence of many concurrent reactions at varying rates should produce very complex kinetics. This does not seem to have been sufficiently recognized. Many inactivation dose-curves have been interpreted by a simpler model, but this may be questioned. In some cases, direct evidence that many amino acids react concurrently has been provided by amino acid analysis (9).

In several cases (6, 7) good protection of enzymes by PABA has been demonstrated. PABA reacts rapidly with OH· radicals and the result therefore indicates that these radicals are causing inactivation. On the other hand, nitrate, which reacts with e_{aq}, afford little or no protection, indicating that e_{aq} may not be causing permanent damage. While protection by radical scavenging seems well established, there is as yet much uncertainty concerning other mechanisms of protection and their relative importance. In particular, there has been much speculation about the role of mercaptan-disulfide interchange in the protection by these types of sulfur compounds; the results of the present work (1, 3, 4) do not support the view that this protection mechanism is very important.

Living cells contain some 60-90% water and the rest is a complex mixture of organic compounds and salts. Therefore, neither the solid state nor dilute solutions adequately represent the natural state of enzymes. That is why it seemed of interest to investigate the behavior of YADH mixed with small amounts of water (8). The results are indeed different from those obtained in the solid state and in solution; a proper interpretation of them is now under study.

Significance of Research: At the present time, the principal connection between ionizing radiation and health is the use of radiation to kill malignant cells in the treatment of cancer. This must be done in a quite empirical way, because we do not understand how radiation causes the death of cells. There is reason to hope that elucidating the mechanism of radiation damage will make possible more rational and effective use of radiation in therapy. This knowledge might also be useful in the control of genetic damage by environmental radiation.

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The aim of the present study is to establish in detail how radiation affects one class of important cellular constituents, the enzymes. The inactivation of enzymes by radiation undoubtedly contributes to the development of radiation injury. Furthermore, enzymes may usefully serve as models for the behavior of other constituents, which cannot be studied as easily and as precisely. An important aim of the project is to find and to evaluate substances that reduce or augment radiation damage. Both types of substances obviously might be employed to extend the applicability of radiation in therapy.

The practical importance of radioprotectors might be underestimated because in ordinary circumstances one quite adequately can prevent radiation injury by controlling exposure. This would not be possible in the case of accidents or acts of war involving atomic devices. One hopes that these possibilities will never be realized, yet as long as they exist there is reason for seeking ways to minimize the consequences.

Publications:

Gorin, G. "The Inactivation of Alcohol Dehydrogenase by X-Rays and the Dose-Modifying Effect of Some Added Substances." Radiation Research 32, 671-684, 1967.

Gorin, G., Doughty, G. and Gideon, R. "Equilibria in Thio-Disulphide Interchanges. Part V. Reaction of Some Aminoethanethiols with 4,4'-Bithiobis (Benzenesulphinic Acid)[1]." Journal of the Chemical Society, Sec. B,(Physical Organic Chemistry) 1967, 729-36.

Gorin, G. and Quintiliani, M. "Inactivation of Aldolase by X-Rays." Experientia, 24:440 (1968).

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00312-04	FACTORS AFFECTING RADIOSENSITIVITY IN MICRO-ORGANISMS	CARL A. BEAM, PH.D. DEPT. OF BIOLOGY BROOKLYN COLLEGE BROOKLYN, NEW YORK

Research Objectives: Some microorganisms display either typically or at certain stages in the cell cycle a non-linear response to radiations, i.e., initial doses are considerably less effective than later ones. In the cases of particular interest this non-linearity has not been associated with normal or transient excesses of sensitive entities within the cells and can therefore be tentatively regarded as the effect of cellular mechanisms which either protect the cells from radiation effects or repair these effects soon after they occur. The present investigation was undertaken in an effort to establish more firmly the reparative or protective nature of this type of resistance, to distinguish between protection and repair, and to elucidate the effective mechanisms.

Progress Report: The investigation employed 50 Kv X-rays and cells of the yeast, Saccharomyces cerevisiae which upon the onset of cell division enter a period of radiation resistance and non-linear response to inactivation which is terminated shortly before division is complete. The essential problem has been attacked in several related ways. Firstly, in the belief that a repair/protective mechanism might be under genetic control, mutants lacking resistance during cell division were sought. Several of these have been isolated and are being studied. To date the inheritance of dividing cell resistance appears to be under the control of at least two genes, damage to each of which is expressed as a simple recessive.

In another approach, the survival of the progeny of cells irradiated in sensitive and resistant stages were compared. Here it was found that the production of some dead cells in post-radiation divisions was less rather than more frequent in dividing cells; i.e. their resistance is not achieved by the segregation of damaged material into one of two daughter cells at mitosis as might be supposed. It was found, however, that a fraction of dividing cells which produce some dead progeny after

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irradiation show an abnormally high recombination frequency upon meiosis. This behavior was not observed in the progeny of resistant cells which did not produce dead progeny, nor in the progeny of irradiated sensitive cells. In one interpretation of these findings it is postulated that an irradiated dividing cell which produces some dead mitotic products also produces occasional chromosomal rearrangements which in turn exert an effect on recombination frequency. Interchromosomal influence of chromosome rearrangement upon recombination frequency was reported in Drosophila melanogaster some years ago, but has to date been found in no other organism. The yeast system is currently under continued investigation with the purposes (1) of challenging the above interpretation and (2) of gaining insights into the mechanism of radioresistance of the dividing yeast cell.

A third approach to dividing yeast cell radioresistance was predicated on the supposition that resistance might result from a repair mechanism that instead of segregating lethal damage into one of two progeny, converted it into non-lethal alteration. In these terms the descendants of irradiated resistant cells would show a higher frequency of the relevant non-lethal alteration than those of sensitive cells. Several non-lethal radiation effects were studied (gene mutation, mitotic recombination, division delay, and petite induction). In all cases the resistant cells resistant to inactivation - were resistant to the non-lethal alteration as well, indicating that none of the non-lethal alterations examined were a by-product of the hypothetical repair mechanism. Instead, since resistance was conferred to cytoplasmic (petite induction) as well as to nuclear events, well separated spacially within the cell, the findings suggest a general internal radioprotection which prevents cellular damage rather than mechanism(s) which repair damage after it occurs.

Other experiments, however, implicate repair as at least one component of the resistance of dividing yeast cells. The response of these cells to an extended series of radiation doses can be divided roughly into two parts - an initial period of non-linearity of response (phase 1) in which each successive dose increment is more effective than the preceding one, and a linear period (phase 11) in which successive dose increments are of equal effectiveness. This rough division can be shown to

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apply to any of the radiation effects studied. One way to regard this type of response is as reflecting of a cellular repair mechanism which is itself ultimately inactivated by the radiation. In these terms resistant cells irradiated within phase 1 are subject to internal repair; those in phase 11 are not. In experiments designed to test this hypothesis it was found that both interdivisional cells and dividing cells in phase 11 of inactivation were subject to a degree of post-radiation mitigation of radiation effects. This mitigation was achieved by shifting the post-radiation nutritional environment from a "rich" one to any one of several which made new demands on the cells' synthetic capacities, as though newly evoked synthetic capacities stimulated or entailed the ability to repair radiation damage. Dividing cells challenged in the same way during phase 1 of radiation response showed no effect of the changed nutritional environment suggesting that the mechanism stimulated in the others is either not sensitive to stimulation at this time or is already operating as a normal component of the dividing cells resistance to radiation. If the second alternative were correct, and in view of the fact that this type of mitigation of radiation effectiveness operates after exposure has ceased, and is therefore reparative rather than protective in nature, it follows that the normal phase 1 component of dividing cell resistance is reparative.

Present evidence thus points to both protection and repair as responsible to some extent for the radioresistance of dividing yeast cells. If ratios of effectiveness are taken from the two phases of dividing cell response and the response of interdivisional cells, the results in general suggest that over 70% of dividing cell resistance results from repair (phase 1) with the remaining 30% (phase 11) referable to protection. The fact that these ratios differ characteristically with the radiation effect measured offers one approach to the elucidation of the mechanisms involved. To date the details of both dividing cell protection and repair remain obscure, and are the subjects of present investigation.

Significance of Research: The biologically mediated resistance to ionizing radiation conferred to the yeast cell briefly during cell division considerably exceeds in effectiveness any other biologically mediated repair or protective system (save

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perhaps that of Micrococcus radiodurans) and any externally administered protective agent. Regarded separately, the tentatively protective phase 11 confers resistance equivalent to total immunity to a number of radiation effects (e.g. recessive lethal induction). If this protection is effected by a metabolite of adequate abundance and stability, its isolation and identification should be possible and could be of considerable radiochemical, biological and even clinical interest and utility. The mechanism operating during phase 1, tentatively termed repair, can negate in some instances twice as much damage as is prevented by protection (phase 11). The basis of this repair, since to date no by product of its activity has been found, could be of considerable biological interest and importance.

Publications: None

RH 00318-03

GENESIS OF POSTIRRADIATION HYPERLIPEMIA IN THE RABBIT

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Research Objectives: The main objective was to elucidate the mechanism(s) responsible for the marked increase in blood lipid levels following lethal irradiation exposure. Because others have suggested that the degree of rise in blood lipid levels could be used as a prognostic sign following radiation injury we investigated the ability of radioprotective agents and other drugs thought to effect lipid metabolism to modify the marked hperlipidemia and confer radiation protection. Finally the effect of X-irradiation upon the activity of lipoprotein lipase (the enzyme thought to be involved in the incorporation of blood lipids into adipose tissue) was studied.

Progress Report: In all these studies male albino rabbits of 2.0-2.5Kg were used and were routinely fasted for a minimum of 8 hours prior to X-irradiation. All irradiated rabbits received 1000 R whole-body ionizing radiation from a 300 KVP GE maxitron operated at 10ma and HVL of 2 mm Cu. The separation and identification of various plasma lipids and their chemical analyses was according to standard procedures. In

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all acute studies the animals were studied at various time intervals throughout the first 24 hours after irradiation since it is known that the onset of plasma hyperlipemia begins approximately 12 hours after exposure. In this way, we studied the animals prior to and during the development of the lipemic episode as regards each of the parameters we evaluated.

It is known that the concentration of all plasma lipids increases after lethal radiation exposure. However, the most pronounced increase occurs in the plasma triglyceride concentration and for this reason we chose to study this parameter of lipid metabolism. To study the effect of X-irradiation upon the synthesis of triglyceride, we studied the incorporation of palmitic acid 1-^{14}C into plasma and liver triglyceride at 2, 4, 16 and 24 hours after exposure to 1,000 R. The results show that at 2 and 4 hours after exposure the specific activity of liver triglyceride was increased approximately 900% above the values observed in the sham-irradiated group while at 16 and 24 hours the specific activity of liver triglyceride was similar in both groups. Also at 2 and 4 hours after irradiation, there was no increase in the concentration of plasma triglyceride nor was there any increase in the specific activity of plasma triglyceride of the irradiated group. At 16 and 24 hours the plasma lipemic response produced by irradiation was manifested by a 500% increase at 16 hours and a 400% increase in plasma triglyceride levels of irradiated rabbits. Since it has been previously suggested that the secretion of triglyceride from the liver to plasma is not disturbed by radiation exposure and since there was no evidence that the newly synthesized triglyceride was found in the plasma compartment it was obvious there was some other factor involved in the genesis of post-irradiation hyperlipemia than the increase in triglyceride synthesis we demonstrated in the early post-irradiation time periods.

It has been known for some time that an injection of heparin during the peak of the radiation-induced lipemic episode will return elevated serum lipoprotein levels to normal. It has recently been demonstrated that an enzyme called lipoprotein lipase (LL) is apparently involved in incorporating plasma lipoprotein into adipose tissue. Heparin is known to activate this enzyme and it is possible that the reversal of plasma lipoprotein levels of irradiated rabbits to normal was due to activation of LL by heparin. In fact, supporting evidence for this claim may be found

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in the report that irradiated rabbits, cleared from the vascular system and artificial lipoprotein emulsion at a faster rate than the sham-irradiated control group. The effect of X-irradiation on the activity of LL has not been established. To study this effect we chose to study the effect of irradiation on LL activity of the interscapular fat pad of the rabbit. It had previously been shown that this adipose tissue site consists of paired fat pads, identical in weight and chemical composition and alike in their metabolic response to fasting and irradiation. Since it is known that fasting alone will inhibit the activity of this enzyme we initially chose to study this parameter in a fed rabbit to minimize the effect of fasting. The results showed that at 2 and 4 hours after irradiation, a time at which peak triglyceride synthesis had occurred and at which no increase in the concentration of plasma triglyceride was evident in irradiated rabbits, the activity of LL was markedly enhanced by irradiation. The increased LL activity was associated with a similar increase in the incorporation of palmitic acid 1-^{14}C. In this experiment the label was injected immediately after radiation exposure and other studies demonstrated that the incorporation of the label into adipose tissue represented the incorporation of palmitic acid-1-^{14}C which had previously been incorporated in lipoprotein. On the other, at 16 and 24 hours after exposure, during the peak of the plasma triglyceride response the activity of LL was markedly depressed in irradiated rabbits and this was associated with a corresponding decrease in the incorporation of the label into adipose tissue. Thus it appears that irradiation caused a biphasic response in the activity of LL and well explains our previous findings.

However, it is known that irradiation causes anorexia and it was possible that the decreased activity of LL was not solely due to irradiation, per se, but in part, to the anorexigenic effect of irradiation. This seemed highly unlikely since the substrate for LL is plasma lipoprotein and our work and that of others showed that at the peak of the lipemic episode the concentration of serum lipoprotein was markedly increased in irradiated rabbits and that fasting did not at all effect the normal substrate for this enzyme. However, to conclusively prove the effect we demonstrated was due to irradiation, we repeated the experiment employing force feeding of both irradiated and sham-irradiated control groups. In this experiment, the results were

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similar to the previous. Irradiation produced a marked increase in the activity of lipoprotein lipase in the early post-irradiation time periods which was associated with increased incorporation of the label into adipose tissue. A. the peak of the irradiation-induced lipemic response there was a marked decrease in LL activity and the incorporation of the label. These data indicate that the acute lipemic episode following lethal whole-body irradiation is due in part to an increased synthesis of triglyceride in the early post-irradiation time periods and a decrease in the activity of LL during the peak of the lipemic response. In fact, it was shown that irradiated rabbits manifested a continued inability to incorporate the label into adipose tissue after the first two hours post-irradiation whereas control animals demonstrated a continual incorporation of the label.

Exposure to 1000 R X-irradiation was associated with a 12% 30 day survival in control rabbits. The administration of 150 mg/kg of aminoisothiouronium bromide (AET) 10 minutes prior to exposure increased the 30 day survival to 67%. However, even though survival was markedly increased pre-treatment with AET was not able to reverse the plasma triglyceride and phospholipid elevations produced by irradiation in the first 24 and 48 hours. There was no correlation between the degree of lipid response and the ultimate survival of the rabbit. G-50® an antioxidant, which has been effective in preventing the fatty liver response to ethanol was not able to confer any radiation protection nor did it reduce the severity of the plasma lipid response. Thus it appears that the ability of radioprotective agents to increase survival is not related to their ability to modify the lipemic response. It further appears that the degree of the lipemic response is not prognostic of the ultimate fate of the animal.

Finally, we evaluated the possibility that irradiation impaired the ability of the animal to catabolize hepatic fatty acids with the result that these were incorporated into triglyceride at a faster rate, secreted into plasma and with the decrease in LL activity that we demonstrated, contributed to the plasma lipemia. To study this possibility, we measured the concentration of blood ketones throughout the first 24 hours after irradiation. The concentration of blood ketones has been successfully used as an index of the hepatic catabolism of fatty acids. In this experiment

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the rabbits were fasted for 8 hours prior to irradiation and throughout the 24 hours post-irradiation. As expected the concentration of blood ketones in the sham-irradiated control group increased throughout. In the irradiated group the concentration of blood ketones increased in a similar manner as the unirradiated control groups. Both groups therefore reflected a similar increase in blood ketones solely as a result of the fasting state and irradiation did not decrease the hepatic catabolism of fatty acid and this parameter was not a factor in the genesis of post-irradiation hyperlipemia.

Significance of Research: The results demonstrate that major factors in the development of plasma hypertriglyceridemia due to exposure to lethal levels of irradiation are an increase in the synthesis of triglyceride in the early post-irradiation time periods and a biphasic response in the activity of lipoprotein lipase. The activity of LL is increased prior to and markedly depressed during the peak of the lipemia of irradiation. A radiation-induced decrease in the catabolism of hepatic fatty acids is not involved in the lipemic episode. A radioprotective agent (AET) was not able to reduce the severity of the lipemia even though it did prolong the 30 day survival. Similarly an anti-oxidizing agent was neither able to confer protection nor modify the lipemic response.

Publications: No additional publications.

RH 00321-05 STUDIES IN RADIATION PROTECTION

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Research Objectives: The objective of the present research program is to clarify the molecular mechanisms of radiation damage to a system and chemical protection from this damage. These studies, conducted on model systems, are designed to provide information which may be used for a quantitative prediction of the rate and mechanism by which damage will occur as a function of the molecular structure of the species exposed to the radiation. Such studies are expected to lead to development of more effective chemical protectors.

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Progress Report: Our research interests are centered on the understanding of radiation damage and protection mechanisms on a molecular level. The studies which are continuing at this time have been focused on the amino acids as simple model systems of more complex biological molecules. We have studied the rate of free radical attack on these species in anticipation of being able to correlate the rate of attack with site of attack and also to predict the rate with biological macromolecules, for which rates may not be measured directly.

Methyl radicals have been produced by the photolysis of acetone in aqueous solutions. The relative rates of abstraction of hydrogen from amino acids and some peptides have been measured. The methyl radical is very selective in the site of attack. Thus, correlation of this rate of attack with the structure of the amino acid will allow us to rank the different sites of attack in the order of their reactivity. Work has presently been finished on glycine, alanine, methyl alanine, valine, leucine, isoleucine, glycylglycine and glycylglycylglycine. These data indicate a strong dependence or rate on the structure of the amino acid side chain. More data must be collected before quantitative predictions can be made.

Hydrogen atoms have been produced by the Co^{60} γ-radiolysis of aqueous solutions of amino acids at pH = 1.0. The rates of H-atom abstraction or addition to the amino acid was measured by competition of the acid with donors and scavengers with known rate constants. The findings thus far have indicated that the rate constant of H-atom abstraction from amino acids ranges from 10^5 - 10^7 M^{-1} sec $^{-1}$ and is strongly dependent on the nature of the side chain. Thus it appears that reaction may occur at several positions on the molecule. All amino acids which contain sulfur or aromatic groups remove the H-atoms by addition rather than abstraction and the rates of these reactions are nearly as fast as the analogous reactions involving hydroxyl radicals and hydrated electrons (i.e., $k \sim 10^9$ M^{-1} sec $^{-1}$). Methionine, phenylalanine, cystine, tyrosine, and tryptophan were the scavenger amino acids studied while species which donated H-atoms only were: valine, leucine, alanine, α-amino-butyric acid, isoleucine, norvaline, serine, thereonine, lysine, glutamic acid, proline, hydroxyproline, and glycine. The data indicate that empirical correlations of rate and

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structure cannot be made with this small sample. Thus a number of molecules with various structures and compositions are under study.

We are presently starting a study of direct decomposition of known radiation protectors and sensitizers in order to compare the products of this action with those encountered in the Co^{60} γ-radiolysis system. The kinds and yields of products should allow a pattern of decomposition to be established which may be used to determine the mechanism of action of these species in a protective situation. The photolysis of cysteamine and cystamine (protectors) is presently underway. These studies will be followed by analogous decompositions of known sensitizers such as penicillamine to ascertain differences in the decomposition mechanisms of these structurally similar compounds.

Significance of Research: Chemical protection from the effects of radiation could be extremely valuable in cases of unexpected radiation exposure such as nuclear accidents or in cases of fallout or space travel where physical shielding is inadequate or impractical. This research is a step in the direction of a better understanding of the basic processes of radiation damage, and protection from this damage by chemical means. From these studies, an evaluation of the importance of direct and indirect radiation effects may be made. These quantitative studies on model systems should clarify the roles of various components of the protector molecule and permit the development of more effective protectors for use in health protection from radiations.

Publications:

Kuntz, R. R. "The Reactions of Hydrogen Atoms with Simple Thiols." Journal of Physical Chemistry, 71, 3343, September 1967.

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RH 00347-10	RADIATION EFFECTS OF THYMIDINE-TRITIUM	HENRY A. MCQUADE, PH.D. DEPT. OF RADIOLOGY UNIVERSITY OF MISSOURI COLUMBIA, MISSOURI

Research Objectives: We are trying to ascertain at the electron microscopic level where RNA (ribonucleic acid) is made in the interphase nucleus of onion seedling root-tip.

The root-tip is used because it contains a cell population of which many will divide. During interphase of mitotic (growth) division the hereditary material (DNA) within the nucleus is most active in producing RNA. It is generally considered that RNA conveys the genetic directives of the nucleus governing growth, secretion, differentiation and other cellular activities to the cell-plasm.

Progress Report: Onion seedlings with the first rootlets 1-3 mm long were incubated in uridine-H^3 (or thymidine-H^3 for comparison) and then prepared for electron microscope autoradiography. Autoradiographs, in this case, consist of sections of rootlets thin enough for electron microscopy which are covered with a thin coat of photographic emulsion. These specimens are put away in the dark for a month or two and during this time beta particles from the tritium label of the uridine-H^3 strike the emulsion layer. Upon development, reduced silver grains appear over areas of the cell where the labeled uridine resided within the cell at the time of its death. The remainder of the photographic emulsion can be removed with lye solution and when dry the specimen may be examined in the electron microscope. Here the reduced silver grains may be related to the chromatin within nuclei of interphase cells.

Electron micrographs of interphase nuclei show a type of chromatin that is electron dense and another that is more electron lucent or diffuse. In calf thymus nuclei, the diffuse material consists of fibers about 200-250 A thick and appears to be continuous with the dense fibers which are 100 A in diameter. A number of investigations have demonstrated that the DNA, which is active in producing RNA, resides in diffuse chromatin

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and when uridine-H^3 is employed as an RNA precursor, autographs show label over the diffuse areas after very short incubations. After 15 or 20 minutes of isotope-free time, or following long incubation periods, the label is often found over dense chromatin; thus, a shift of label from diffuse to dense chromatin seems to occur.

We have been studying the meristem cells of onion root tip and have literally examined hundreds of thin sections of interphase nuclei. These cells which are more or less undifferentiated seem to behave somewhat differently than those of other investigations in that five minute incubations in uridine-H^3 with no subsequent cold chase have provided autographs in which label is found largely over the dense chromatin. With longer incubations and with various isotope-free periods following, the label appears as expected over the dense material. The fact that label does not seem to shift from diffuse to dense chromatin interests us and we are currently studying the results of more short incubations. We know that the label we see in our autoradiographs is that of RNA because we can remove label by the use of RNAase, an enzyme which specifically attacks RNA. We have demonstrated this by using sections thick enough for phase microscopy where we have floated control sections in water and others in RNAase solutions; in the latter, no label appears while label is present in water-treated sections. We have also treated electron microscope sections enzymatically and removed virtually all label from uridine-H^3; this does not happen in water controls.

It is possible that these relatively undifferentiated rootlet cells, many of which will enter mitosis, behave differently than the cells which have been studied heretofore (calf thymus nuclei, cultured mammalian cells, cells of Triturus); it is also possible, of course, that RNA synthesis occurs so fast in rootlet cells that we have simply been unable to observe label over diffuse chromatin and that the shift does actually occur very swiftly. The study is of interest to us because it is concerned with the state of chromatin, i.e., chromatin may be RNA synthetic or not depending on its disposition. How and why the disposition changes may be related to gene action.

Significance of Research: Molecular biology has elucidated the structure and much about the behavior of the DNA molecule. Our morphological concept of the chromosome

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has been derived on the one hand from the observations of optical (light) microscopy and on the other from transmission genetics. Between the two bodies of knowledge, one founded on molecular biology and the other on classical cytogenetics, is a great gap. We know the DNA molecule, and we know something of other chromosomal components, but how are all these put together and how can we go about constructing an operating model of a chromosome?

There are several investigational methods used in efforts to fill the gap. At the macromolecular level, studies of chromosome organization are done with the electron microscope. At the physiological level, cytochemical studies can tell us something about chromosomal behavior from which we might draw inferences about the chromosome's ultimate structure. It is information of this sort which can help us to understand the behavior of the genetic material in health and disease. Within the realm of interesting speculation about future health progress is the idea of altering or repairing unfavorable genetic material or genetic lesions. Obviously any knowledge of chromosome structure would be basic to such efforts.

Publications: None

RH 00358-04 EFFECTS OF HIGH ENERGY RADIATIONS ON LIPIDS

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Research Objectives: The objectives of the project are to study some of the compounds formed as a result of irradiation of lipids, to determine the effects of irradiation on the physical and chemical properties of complex lipids such as phospholipids and lipoproteins which are important constituents of cellular membranes, and to investigate what secondary effects lipids may have on irradiation damage of other essential constituents of biological tissues and upon changes in normal biological reactions brought about by irradiation.

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Progress Report:

a. Equipment. An all-glass apparatus has been constructed that makes it possible to collect volatile compounds from irradiated lipids under high vacuum and at room temperature. The apparatus is equipped with several condensing traps which can be kept at different temperatures down to that of liquid nitrogen. The condensed material can be sealed directly in the traps under vacuum and, by means of break-seal side arms, it can be transferred later, with essentially no loss or exposure to oxygen, to other apparatus for further fractionation and analyses.

Sample holders compatible with the size and geometry of the 9.5 kilocurie cesium-137 source at the University of Minnesota Gamma Irradiation Facility have been designed and constructed. The rotating sample holder is located in the center of the source and permits irradiation of up to 35 samples at one time at a uniform dose rate to insure that all the samples are treated uniformly.

b. Formation of polymers during irradiation. Highly purified methyl esters of simple fatty acids were irradiated under vacuum or in an atmosphere of oxygen at doses of 2 to 20 megarads. The extent of polymerization in the irradiated sample was determined by analytical micromolecular distillation and the samples were fractionated at the same time into monomers, dimers and higher polymers. The fractions were examined by ultraviolet, infrared and near-infrared spectroscopy and by determinations of molecular weight, unsaturation, epoxy groups and peroxides.

No polymers could be detected in methyl myristate, stearate, oleate, elaidate, linoleate or linolenate irradiated with up to 10 megarads under vacuum. Even with 20 megarads no polymers were found in methyl oleate, but about 0.5% dimer and higher polymers were detected in methyl linoleate and linolenate. These amounts, however, were too small for further investigation.

Irradiation under oxygen gave only traces of polymers in methyl oleate after a dose of 20 megarads, but approximately 4% of dimer and higher polymers were isolated from methyl linoleate or methyl linolenate treated in the same manner. More dimers were

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found in irradiated methyl linoleate, while the linolenate contained proportionately more of the higher polymers.

Very little peroxide was found in methyl oleate irradiated with 20 megarads in the presence of oxygen. Irradiated methyl linoleate had a peroxide value of 437 meq./kilo, while the peroxide content of irradiated methyl linolenate was only 351. Although linolenate oxidizes more rapidly and to a greater extent than linoleate, linolenate peroxides are more easily destroyed by irradiation, and this results in a greater accumulation of peroxides in the linoleate. In both cases, the peroxides were found mostly in the monomers, while the dimers and the higher polymers contained only small amounts of these compounds.

Determination of molecular weights, unsaturation and conjugation indicated that the linoleate and linolenate dimers were not of the cyclohexyl type resulting from a Diels-Alder addition. Mass spectrometry on a fraction of the linolenate dimer supported these observations and indicated further that the dimers consisted of two methyl ester residues linked together by a single carbon bond. No oxygen linkages could be detected in these compounds. The formation of larger amounts of dimers during irradiation in presence of oxygen, therefore, is not due to the formation of oxygen linked dimers, but probably to the catalytic effect which peroxides have on dimerization.

c. Effects of lipids and irradiation on enzyme activity. Although it is difficult to completely destroy enzymes by irradition, there have been many reports of decreased activity following irradiation of some enzymes, while in other cases, increased enzymatic activity has been reported following irradiation of tissues. Because lipids are attacked easily by radiations and form highly reactive free radicals, it was thought they might have some effect on the changes observed after irradiation of enzyme systems.

Three enzymes were selected for this study: glutamic-oxalacetic transaminase (GOT) is easily released from muscle tissue, particularly heart muscle, into the blood

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stream as a result of tissue damage, and is used clinically as a measure of heart damage following myocardial infarcts; malic dehydrogenase (MDH) is an important electron transport enzyme in the tricarboxylic acid cycle; and alkaline phosphatase is of major significance in lipid metabolism. The fatty acids selected for study were the cis-cis, cis-trans, trans-trans 9,12-octadecadienoic acid isomers.

Considerable difficulties were encountered in preparing enzyme-lipid systems that remained homogeneous and in which the enzymes retained their activity long enough to complete the study. It was desirable to use methyl esters rather than soaps to avoid the higher pH required to keep soaps in solution and, also, to avoid introducing additional cations into the system. However, this was possible only with alkaline phosphatase. With the other two enzymes, the concentrated ammonium sulfate solution required to maintain their activity did not allow the formation of a stable emulsion. In these cases, a 5% dispersion of potassium soap in the enzyme solutions was used.

The enzyme-lipid preparations were irradiated in the presence of air in open tubes. Double controls (i.e., enzyme + lipid, non-irradiated and enzyme-irradiated without lipid) were run at the same time.

With alkalin phosphatase the presence of the methyl esters during irradiation gave considerable protection to the enzyme against irradiation damage. Without lipids, the enzyme was completely destroyed after irradiation with 2 million rads, and 90% of the activity was lost with a dose of 200,000 rads. In presence of methyl esters, however, 45% and 76% of the activity remained after irradiation with 200,000 and 2 million rads, respectively. The three linoleate isomers were equally effective in protecting the enzyme and they had no effect on enzyme activity when used alone in non-irradiated controls.

On the other hand, the activity of glutamic oxalacetic transaminase remained unaffected by irradiation or the addition of linoleate soaps either separately or in conjunction with each other. This enzyme under the condition of the experiment appears to be completely resistant to irradiation damage at doses up to 2 million rads.

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We were unable to determine satisfactorily the activity of malic dehydrogenase under carefully controlled conditions in several samples of enzyme preparation and, for this reason, studies with this enzyme had to be abandoned.

Significance of Research: The cause and mechanism of irradiation damage to living tissue remain largely unknown. Purely on a random basis, the small doses of high energy radiations that are sufficient to cause serious damage to man and other higher animals would be expected to affect directly relatively few molecules of the very abundant protein and lipid tissue constituents, and, much less yet, the scarcer but more vitally important components, such as the vitamins, hormones and enzymes. The effect of irradiations, therefore, must be due to either selective direct attack on essential molecules that control vital metabolic processes or to secondary reactions of the irradiated more abundant tissue constituents with these essential components.

Polymers and cyclic monomers resulting from autoxidatior and from heat treatment of edible fats and oils have frequently been reported to be toxic. Furthermore, polymers are formed readily when certain fats such as methyl linoleate are irradiated in the solid state. Apparently the oriented packing and immobilization of molecules in a crystal structure facilitates greatly irradiation polymerization. Lipid molecules are also closely packed and oriented in cellular membranes and one may wonder if such molecules are susceptible to radiation polymerization also. Polymerization or cross-linking of fatty acid molecules in membranes would be expected to change drastically the permeability and other characteristics of cell walls. Thus, a knowledge of the type of polymers resulting from irradiation of lipids and of the conditions under which they are formed may be of some importance in understanding the effect of radiations on living tissues.

Irradiation of polyunsaturated fatty acid molecules gives free radicals which, under the oxidizing conditions existing in tissues, probably form peroxides and may initiate chain mechanisms that generate new free radicals. A number of molecules with reducing properties, such as those containing SH groups and hydroquinoid structures, are known as free radical acceptors, and by destroying or by reacting with the free radicals

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resulting from irradiation, these compounds act as protectors against radiation damage. It is known, also, that hydroperoxides react readily with amino groups and, thus, there are many possibilities for secondary reactions between lipid free radicals and their oxidation products and other components of tissues.

On the other hand, the ease with which polyunsaturated lipids are attacked by irradiation may cast them in the role of radiation protectors with respect to some other molecules. Perhaps an example of this is the protection of alkali phosphatase which has already been discussed. However, this by no means suggests that all enzymes which are sensitive to irradiation will be affected the same way when lipids are present.

The role which lipids may play in bringing about changes in living tissue as a result of irradiation remains largely unknown, although it may be of some significance in the areas of irradiation injury and irradiation therapy.

Publications: None

(1) RH 00367-03 BIOLOGICAL MECHANISMS OF RADIATION DAMAGE

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Research Objectives: The primary interest of this program is to locate and describe the biochemical changes which are associated with and responsible for the physiological changes which are characteristic of radiation damage.

We have previously observed, in the rat, that there are marked changes in the functional activity of the digestive tract, the cardiovascular system, and the central nervous system following total body irradiation with 750 R (LD_{50} dose) of X-rays or cobalt gamma radiation. Some of the more marked changes include a loss of ability of the small intestine to transport electrolyte ions against concentration gradients, changes in the electrocardiogram, venous pressure and chemical composition

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of the heart, and the characteristic pattern of changes in the brain waves and in the chemical composition of the brain. All of these characteristic effects occur some hours or days after radiation exposure. The biochemical and structural changes which occur in the intervening period and which lead to these functional changes are not known, but are our central interest in this project.

Progress Report: An LD_{50} dose of radiation releases only a minute amount of ionizing energy within the body. In order for this to have a major functional effect, it seems necessary that this radiation must have an effect upon certain enzyme systems. By affecting a catalyst, the overall destructive effect can, with time, be greatly magnified. Furthermore, since the characteristic effects of radiation are most evident several days after radiation, it would seem possible that the primary effects may be upon the mechanism of enzyme formation (the DNA system), upon the lysosomal system (which is normally responsible for the autolysis and removal of cells damaged by trauma, infection, etc.) or upon the cell membranes (and thus affect the homeostatic mechanisms which are responsible for maintaining a normal intracellular fluid and electrolyte environment within the cell). It is possible that any one or that several of these mechanisms may be responsible for the changes observed in certain organs. Certainly all three of these mechanisms need to be studied.

We have reported the quantitative changes that occur in DNA in all of these organs and systems and have provided one of the earliest reports that radiation directed to one part of the body has a major effect upon DNA synthesis in other parts of the body. The biochemistry of the DNA changes has been extensively studied in other laboratories. Work with this system is of genetic interest but, up to now provides little hope for practical therapeutic result in regard to the treatment of persons exposed to radiation. For these reasons, our immediate interest has been directed toward the study of the lysosomal system and the study of the fluid and electrolyte changes within the different organs of the body.

Bruises and burns are among the stresses which result in direct damage to cells. The damaged cells are then destroyed and removed by the action of the lysosomal system.

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The lysosomes are particles within the cell that contain high concentrations of a variety of hydrolytic enzymes. It is usually pictured that the rupture of the lysosomal membrane releases these enzymes directly into the cytoplasm of the cell, and autolysis ensues. Our work with the lysosomal system has shown that the release of these enzymes is considerably more selective than this. It is not an "all or none" effect. We have described (in our publication "Effects of Whole Body Gamma Irradiation upon Lysosomal Enzymes in Rat Tissues") at least four different groups of lysosomal enzymes that are released to different degrees at different specific times following irradiation. Presumably different release mechanisms are involved. The extent and timing of this destructive hydrolytic action varies considerably from one organ to the next. We have also studied the action of other types of hydrolytic enzymes within the cell (paper submitted). These are not part of the lysosomal system and act in a different fashion. There is no single or simple pattern of hydrolytic enzyme change which occurs throughout the body following irradiation.

One of the major problems in radiation biology is to discover why certain organs and systems are affected to different degrees and at different periods following radiation. Within the wide variety of patterns of lysosomal and other hydrolytic enzyme changes which we have described one can find a group of statistically significant biochemical changes which offer differences with respect to organ and with respect to time after irradiation. This system needs further study.

Vomiting, diarrhea and other fluid and electrolyte changes have long been recognized as among the most characteristic symptoms of radiation sickness. The problem that we have sought to investigate is the fluid and electrolyte changes which occur at the cellular level. To do this requires that one obtain, simultaneously, two sets of experimental data. The first of these relates to the fluid and electrolyte concentration within a given tissue sample. The other (an estimation of extracellular fluid volume) allows one to estimate the amount of the measured fluid and electrolyte which is outside of the cell. By difference, one estimates the amount of fluid and electrolyte which are within the cell. To determine the total water and electrolyte

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(sodium, potassium and chloride ions, in particular) in a given tissue sample is a relatively straight forward analytical chore. But to determine the extracellular fluid volume of a tissue has been a highly uncertain matter. We used six different methods. These agree reasonably well under normal conditions (paper submitted), but under the stress of radiation, the agreement is so poor as to leave serious doubt concerning the interpretation of any intracellular fluid and electrolyte data computations. On the basis of a statistical study (in preparation), we have discovered a pattern to the disagreement between these different methods. We have described the reason for the errors observed in certain of the methods, and have provided a means for estimating the extracellular fluid volume that avoids this error under these experimental conditions. This new method is a major contribution to the field of fluid and electrolyte metabolism. It also provides data on cell composition not previously available in radiobiology.

On the basis of this new methodology, we have been able to demonstrate that there are:

a. marked and consistent changes in interstitial fluid (concentrations change in a parallel fashion in all organs throughout the body);

b. increases in intracellular sodium ion concentration which occur in key organs just prior to functional changes observed in these organs;

c. no changes in intracellular water concentration in any of these organs at any time.

It is known that certain intracellular enzymes are sensitive to and are inhibited by small increases in sodium concentration. The relative sodium and potassium concentration gradients across all membranes are also importantly correlated with their electrical properties.

Significance of Research: The results of this work do not provide final answers to all questions in radiation biology, but they do bring us several steps closer to

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understanding the long progression of intracellular changes that lead to the delayed functional changes which occur within the gastrointestinal tract, the heart, and the brain of the irradiated animal. The changes in lysosomal enzyme activities and the changes in intracellular electrolyte concentration, in theory at least, are factors that are more susceptible to therapeutic control than might be true in the case of changes in the DNA system.

By administration of standard doses of X-rays, one can produce large and repeatable physiological changes in the gastrointestinal, cardiovascular and central nervous systems. Parallel with these are corresponding changes in the chemical composition of these tissues. This provides a useful tool for those interested in further studies of biochemical-physiological relationships, and by other workers interested in lysosomal enzymes, fluid and electrolyte metabolism, digestion, electrocardiography, etc. The results of such work can provide not only the basis of further growth in these collateral fields, but can provide the basis for extensive later interpretation of the radiation effects that we have studied. The interrelationship between fields is important to the development of practical therapeutic methods. For example, it is known that certain hormones, and that the dietary intake of certain vitamins, can have a marked effect upon the release of certain lysosomal enzymes. We have shown that there are at least four distinct and independent lysosomal enzyme release mechanisms operative in the post-irradiation period. The control of any or all of these could have practical effects in relation to the treatment of accidentally or therapeutically irradiated individuals.

The more closely one can correlate specific functional changes (electrocardiogram, heart sound, blood pressure, electrocorticogram, etc.) to a well defined set of biochemical changes occurring in specific portions of the body, the greater the opportunity one has to understand and control body changes.

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RH 00367 (Continued)

Publications:

Noaman, M., Hamdy, M. K. and Caster, W. O. "Effects of Whole Body Gamma Irradiation Upon Lysosomal Enzymes in Rat Tissues." Ga. Acad. Sci. 26: 10-23, 1968.

Caster, W. O. "Intracellular Changes in Rat Heart After 700-R Total-Body X-Irradiation." Abstract Fa-12, Radiation Research, Vol. 31, No. 3, July 1967.

RH 00379-03 RADIATION CHEMISTRY OF SULFUR BIOCHEMICALS

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Research Objectives: We chose to study sulfur compounds for several reasons. They were known to be rather sensitive to radiation; many of them are critically important in living systems; sulfur groups hold many proteins in the right shape for biological activity; and sulfur compounds comprise the best known group of radiation-protective agents. By studying simple chemical systems at low doses of radiation, we hope to discover underlying principles which might indicate what is really going on in the enormously-more-complicated living organisms.

Progress Report: Our work has revealed two fundamental phenomena which, we feel, are of crucial importance in radiobiology.

a. When simple, and even moderately complex disulfides are exposed to X-rays in dilute solution in water, radiation damage occurs at sulfur exclusively, even with substances whose structures contain even dozens of other potentially radiation-sensitive atoms and groups. The biochemically important disulfide oxidized glutathione, for example, contains two sulfur and seventy other atoms, yet, as long as unmodified disulfide remains, no other atoms, or groups are attacked. This is rather like firing a machine gun into a basket containing many white eggs and a few brown ones, and finding that only brown eggs are smashed! We can rationalize this, however, if we note that a solution of glutathione (or anything else, for that matter) in water is mainly just

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water. Our basket, in effect, contains not only eggs but a thousand steel balls (water molecules), for every egg. Bullets will almost always hit steel balls. If the impact fragments a ball into thousands of tiny pieces of shrapnel, each barely big enough to crack an egg, and if the brown eggs (sulfur) have considerably weaker shells than white (other atoms) then only brown eggs will be demolished.

Confirming this picture, we find the number of sulfur groups oxidized (brown eggs smashed) is never greater than the number of reactive species (pieces of shrapnel) generated in the system, no matter how many molecules of sulfur compound (brown eggs) are present.

This phenomenon clearly indicates that, even though other radiation-sensitive sites may be present in a living cell, damage will be directed quite strongly towards sulfur atoms of sulfur containing molecules such as proteins.

b. A Special variety of disulfides, the mixed and cyclic disulfides, undergo an additional reaction, scrambling or cross-linking, upon irradiation. The amount of this reaction is not limited by the number of reactive species (free radicals) generated by the radiation; it can be many times larger and depends on the concentration of the disulfide. Pushing our analogy to the limit (perhaps beyond), it is as if each brown egg were precariously balanced on its small end, and this reaction simply involved knocking it over. If few such eggs are present (low concentration) an egg, hit, would simply smash as before. But if many are present, close together, it is possible for the first, in falling, to knock over a second, and it a third, and so on. The more such eggs there are, (higher concentration) the more will be knocked over as a result of a single initiating event. We must not push the analogy too far, since we might have to start suggesting reconstitution of an egg by a second hit. Suffice it to say that a kinetic theory which we have developed rationalizes these novel experimental observations quite nicely.

Many proteins, essential constituents of living cells, are special cases of mixed, cyclic disulfides containing a very high concentration of disulfide units

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within each molecule. From our data, we can predict that a single reactive radical (one of thousands generated by a single X-ray photon absorbed) colliding, by chance, with such a molecule would cause scrambling of all the disulfide groups. The results of such scrambling are well known from other work; the protein loses all biological activity and becomes, effectively, dead.

Significance of Research: One example, only, is given. The genetic material, DNA, of the chromosomes of a cell is embedded in a massive sheath of protein. The sheath protein is known to contain much disulfide sulfur; scrambling of this would cause genetic malfunction just as surely as would direct attack on the genetic material itself, since it would prevent proper unfolding and exposure of the DNA. Many thousands of reactive fragments (free radicals) are generated by every particle of radiation energy absorbed by a cell. The possibility that any of these would pass unconsumed through the massive, reactive protein sheath and react directly with the DNA is very remote. But a single one, generated anywhere in the vicinity of the sheath, will very probably react with sulfur and scramble hundreds of disulfide groups. The sheath will, in effect, lock solid. Since an individual cell usually contains only one of each specific chromosome component, an essential biochemical characteristic may be irretrievably lost as a result of a single, highly probable, event. The cell will malfunction and probably die.

Radiation-protective drugs, then, should:

(1) Pass, unchanged, into cells; be non-toxic; be heavily absorbed onto sheath protein and related structures.

(2) React exceedingly readily with free radicals giving, in turn, radicals which either destroy themselves or are so unreactive that they will not react with disulfide.

Many of the known protective compounds fit these requirements.

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Publications:

Owen, T. C., Rodriguez, M., Johnson, B. G. and Roach, J. A. G. "The Radiation Chemistry of Biochemical Disulfides. I. The Low-Dose X-Radiolysis of Cystine." Journal of the American Chemical Society, 90, 196, 1968.

Jayson, G. G., Owne, T. C. and Wilbraham, A. C. "The Radiation Chemistry of Cystamine Sulphate." Journal of the Chemical Society, 1967.

Owen, T. C. "Amino-alkanethiols from Amino-alcohols via Aminoalkyl Sulphates and Thiazolidinethiones." Journal of the Chemical Society, Section C, 1967.

(1) RH 00403-03 — IMMUNOGENETIC STUDY OF RADIATION BONE MARROW THERAPY

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Descriptor: The aim of this project is to elucidate further the dynamics of hemopoietic repopulation following lethal total-body irradiation and bone marrow transplantation. The information resulting should be valuable in studying basic concepts of immunogenetics.

Publications: None

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(1) RH 00405-02	ESR STUDIES OF CONJUGATION BY SULFUR	ROBERT G. HAYES, PH.D. DEPT. OF CHEMISTRY UNIVERSITY OF NOTRE DAME NOTRE DAME, INDIANA

Research Objective: The idea of conjugation, which means the tendency of a certain type of chemical bond called a pi bond to spread itself over a molecule rather than be localized between two atoms, is one of the important theoretical concepts of chemistry. This research is directed toward the measurement of the extent to which a sulfur atom in a molecule engages in conjugation. The experimental method chosen, electron paramagnetic resonance, permits one, under favorable circumstances, to map out the distribution of an electron in a molecule.

Progress Report: As indicated in the last paragraph, we are using electron spin resonance to assess the extent of conjugation of the sulfur atom. Since the use of electron spin resonance requires the presence of unpaired electrons in a molecule most molecules are not suitable. It is often possible, however, to create a species with an unpaired electron from an ordinary molecule by adding or subtracting an electron, using chemical means or electrolysis.

We have studied various compounds containing divalent sulfur, of the type R-S-R', and also several sulfanes, of type $R\text{-}SO_2\text{-}R'$, and a few sulfoxides, R-SO-R'. The work consists in preparing radical ions of the compounds of interest, obtaining and analyzing their electron spin resonance spectra and comparing the results with the predictions of approximate theories of bonding. We have used a very simple theoretical description of bonding called Huckel theory. The final result of the work is, then, some numbers which one may use in the theory to obtain the bonding. These numbers are, thus, a measure of the extent of conjugation.

In brief, there seems to be very little conjugation in compounds of the type R-S-R', in the sense that the electrons already on the sulfur atom show very little tendency

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to spread onto other adjacent atoms, forming double bonds (this is called p-type conjugation) and, besides, there is no evidence for the existence of a tendency for electrons on adjacent atoms to spread onto the sulfur, which is called d-type conjugation. The latter is particularly important because it has been supposed that d-type conjugation is important in sulfur on the basis of conflicting chemical and spectroscopic evidence.

The sulfane grouping, $-SO_2-$, is probably restricted to d-type conjugation and the evidence is that this conjugation is fairly strong. Problems have arisen, however, in deciding just how much conjugation exists. There are two compounds which require different sets of parameters, in the approximate theory, to reproduce the observed spin distributions. The experimental assignments have been verified, in one case by us, in the other case by the worker who first observed the spectrum. The anomaly is at present unexplained.

We have, in the course of the work, also obtained a description of the sulfur atom in a radical of the type R-S', in which the sulfur atom is bonded to only one other atom. A radical which appears in the course of the oxidation of thiophenol can, by an argument based on the appearance of the spectrum and the expected mechanism of the reaction, be assigned to a sulfur-containing species of the type R-S'. This is interesting because no information on the bonding in such systems has been available until now and radicals of this type are probably more important in radiolysis than those of the type with two bonds to sulfur.

Significance of Research: This research bears on the ability of sulfur-containing compounds to provide protection against the effects of radiation. It has been known for some time that such a protective effect exists and it is supposed that the protection is due to the localization of radiation damage in sulfur radicals, of the type R-S', presumably because they are quite stable. This research starts to attack the problem

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from the other end, beginning with the properties of a sulfur atom in a compound. It would appear that we now have sufficient information, on the basis of this work and others, to understand the stability of sulfur-containing radicals.

Publications: None

RH 00422-03

PHOTOENZYMIC REPAIR OF RADIATION-DAMAGED NUCLEIC ACID

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Research Objectives: This research is directed at the detailed mechanism of the photoenzymatic repair process which counteracts damage done by ultraviolet radiation to the genetic material (DNA) in many types of cells. It aims to describe the repair steps as chemically defined events. It also encompasses whatever study is required of the biological systems used for detecting DNA damage outside cells in order to employ these as an effective tool.

Progress Report:

A. This work has employed the bacterial transformation process (in which genetic information is conveyed to cells of Haemophilus influenzae by purified DNA extracted from mutant strains of the same species) to detect the presence or absence of damage in this genetic material. Biologically significant damage to the DNA diminishes its transforming activity; repair will then enhance it. We have investigated the way in which inactivation depends on such damage, and shown through theoretical models of the transformation process how to relate observed loss of biological activity to the amount of primary damage.

Cells undergoing transformation by damaged DNA partly repair it after uptake, thereby diminishing the observed effects of radiation damage. The amount of such intracellular repair differs for transformation with respect to different genetic traits

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(genetic markers). We have been able to describe the circumstances under which repair is found to be high or low in one particular region of the H. influenzae genome (collaboration with Research Associate Rufus Day). These strongly suggest that the degree of homology between the incoming DNA and the corresponding region of the recipient cell genome plays a decisive role.

We have also succeeded in isolating two repair deficient mutants of H. influenzae for clarifying repair effects in the transformation assay.

B. Using the transformation assay we have undertaken detailed study of the reaction producing photoenzymatic repair (photoreactivation) of ultraviolet radiation damage to DNA. We showed earlier that the photoreactivating enzyme (PRE) from yeast combines with a site of ultraviolet damage in DNA, and that this enzyme-substrate complex then absorbs a light quantum to initiate the repair.

We have used high intensity flash illumination to measure the rate of formation of the complex (collaboration with Research Associate Helga Harm). A flash given immediately as PRE and irradiated DNA are mixed produces essentially no recovery, but later, as the complex forms, it does. The extremely low concentration (in the chemical sense) of the reactants makes this formation quite slow: $\sim$10 minutes for maximum effect. Use of paired flashes with varying time between also shows effects expected from a highly non-uniform damage concentration, sites being clustered on DNA molecules which are relatively far apart. These phenomona must be recognized in designing experiments with the system.

Flash illumination at high enough intensity causes repair of essentially all damage sites complexed with the enzyme. Under some conditions this permits measurement of numbers of repair enzyme molecules relative to damage sites. If numbers of damage sites are assumed equal to the numbers of pyrimidine dimers found in irradiated DNA

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this permits an absolute count. By this estimate PRE constitutes about 1/1,000,000 of the protein in a crude extract of yeast. Analogous studies on living cells (collaboration with Prof. Walter Harm) indicate about 20 PRE molecules per bacterial cell in Escherichia coli.

Under conditions of large enzyme excess and continuous illumination only the light-dependent step affects the rate of repair. We have measured the amount of damage in DNA from the biological effect during repair with excess enzyme, and found that in its rate of disappearance it behaves not like a single homogeneous damage but like a mixture of two or more which are repaired at different rates. Moreover, the action spectrum (the relative effectiveness of different wavelengths of light in driving this repair) is different when using different degrees of recovery in the measurement. This is not surprising considering the complexity of DNA structure, but makes it desirable to concentrate the primary study on damage in synthetic DNA's of particularly simple composition.

We earlier developed a method for detecting such damage in synthetic DNA (which lack biological activity) by observing the competitive inhibition of transforming DNA repair when irradiated synthetics are present in the same reaction mixture. The method has now been improved to permit single tests on samples of as little as 1/10,000,000 gram. (This will permit an extensive experimental study with several scarce polymers which we have hitherto not been able to undertake.)

We observed the action spectrum for photoenzymatic repair of the polydeoxy adenylate-thymidylate polymer dA:dT (where the damage occurs in thymine). This is different from the action spectrum for the repair in polydeoxyguanylate-cytidylate dG:dT (where the damage occurs in cytosine). This difference proves that light absorption by the enzyme-substrate complex does not depend on the enzyme alone, but is at least partly determined by the substrate combined with it. Taken with other known information, this fact argues that PRE itself does not possess a structure absorbing light in the effective region, and that absorbance in the complex is essentially a modified absorbance of the substrate.

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We have shown photoreactivable UV damage in natural DNA or dA:dT exposed to light of wavelength 3341Å in the presence of dilute acetophenone (p.01 Molar), but this does not occur in dG:dT treated the same way. (In no case does 3341Å light do anything without the acetophenone.) This is understandable on the basis of studies by others on energy transfer from excited molecules of acetophenone to the molecular sub-units of which DNA is composed. It is thus possible to create the damage preferrentially in some structures of DNA (thymine sequences) while not others (cytosine sequences) where it would be formed by ultraviolet.

Significance of Research: The hazard to a biological system presented by a particular radiation exposure can be alleviated by any measures which (1) diminish the initial damage caused by this exposure and (2) increase the capabilities of the biological system to recover from such damage.

Radiation damage is a result of high energy excitations of biological molecules and the resultant chemical changes in certain critical structures -- principally the genetic material, DNA. Cells have processes for repairing this damage. We are principally concerned with understanding the detailed mechanism of one of these repair processes, which alleviates injury by the ultraviolet radiation of natural daylight, somehow utilizing molecular excitations by longer wavelength light. In such a study we must also deal with light-independent repair mechanisms occurring in the same cellular systems which can repair damage by both ionizing and ultraviolet radiation. The results should contribute to general understanding of damage and repair processes.

Publications:

Rupert, C. S. "Shapes of the U.V. Inactivation Curves for Single and Linked-Double Markers in Haemophilus Influenzae Transforming DNA." Photochemistry and Photobiology, Vol. 7, pp. 437-449, 1968.

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RH 00422 (Continued)

Publications:

Werbin, H. and Rupert, C. S. "Presence of Photoreactivating Enzyme in Blue-Green Algal Cells." Photochemistry and Photobiology, Vol. 7, pp. 225-230, 1968.

Day III, R. S. and Rupert, C. S. "Effects of Genetic 'Mismatch' on UV Sensitivity of H. Influenzae Transforming DNA." Biophysical Journal 8 A51, Abstracts of Twelfth Annual Meeting, 1968.

RH 00429-03 CHELATION IN RADIATION PROTECTION CHEMICALS

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Research Objectives: The broad objective of this project is to better understand the mechanism of chemical protectants against ionizing radiation and to be able to produce more effective and useful materials. More specifically, it is planned to synthesize and study the effect of protectant compounds that are capable of chelation. The effect of chelation on radiation protection is being investigated by producing a series of compounds that have the same reactive structure and are known to chelate and to be radioprotective, but to introduce substitutents in the nonreactive part of the molecule, which will influence the electrical properties and thereby the ability to chelate.

Progress Report: Several series of organic compounds which have the ability to chelate were prepared in the laboratory. All of the compounds contained a benzene group which could be substituted in the para position so as to influence the strength of the chelate by the inductive effect of the ring. Compounds of the following series were made:

1. Dithiocarbamates
2. Thiocarboxylic Acids
3. Sulfinylcarboxylic Acids
4. Phenyl Mercaptoalkyl Amines
5. Mixed Thiolsulfonates

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The compounds that were synthesized were evaluated for radiation protection employing E. coli bacteria as the test organism. The toxicity of the compounds to the bacteria was first determined so that the highest safe concentration weight be employed in the radiation protection test. The compounds were dissolved in a suitable solvent and diluted with water to the maximum non-toxic concentration and the bacteria were added. The mixture was exposed to X-rays for varying lengths of time and the number of surviving bacteria were determined as compared to a solvent control without the compound, similarly treated.

The first group of compounds to be tested were the sodium para-substituted N-phenyl dithiocarbamates. These were selected because of the work of Van Bekkum et al (1956) that found the N-phenyl dithiocarbamate ($x\text{-}C_6H_4\text{-}NHCS_2Na$) to afford significant radiation protection to mice. We prepared and tested the phenyl, the p-OH, the p-CH, and the p-Cl compounds. Also tested were sodium dimethyl dithiocarbamate and sodium diethyl dithiocarbamate. Surprisingly enough, none of these compounds showed protection. In fact, many of them were sensitizing; that is they caused greater destruction of bacteria than samples exposed to X-rays without the compound, and greater than with the compound but not exposed to X-rays. The more electronegative (electron withdrawing) the groups on the ring (Cl,H) the more sensitizing the compound, and the more electropositive (electron donating) the groups (OH, CH_3) the less sensitizing the compounds. None of the N-phenyl dithiocarbamates were protective against radiation.

In the case of the para-substituted phenyl acetic acids, ($x\text{-}C_6H_4\text{-}SCH_2CO_2H$), the same order of radiation protection activity versus group substitution in the ring occurred as with the N-phenyl dithiocarbamates. That is, the electron withdrawing groups on the ring (NO_2, Br) gave compounds which were radiosensitizing whereas the election donating groups (CH_3,NH_2) gave compounds which were radioprotective or neutral. With p-substituted phenyl propionic or butyric acids there was little or no incluence on biological activity as a result of the substituents.

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The p-substituted S-phenyl mercaptoethyl and mercaptopropyl amines showed some protection in some cases but an insufficient number of compounds were tested to make generalizations regarding the effect of the substituents. It should be noted that in all these compounds the size of the chelate ring influences the ability to chelate and the nature of the substitutuent in the aromatic ring influences the strength of the chelate.

A few mixed thiolsuifonates were tested. Of all the compounds we have made, cysteinyl p-toluene thiolsulfonate gave the greatest radiation protection. Unfortunately it is quite toxic to microorganisms and therefore cannot be employed at high concentration, but it is remarkable that it gives protection at such low concentration. It may be possible to vary the molecule so as to lower the toxicity without destroying its ability to protect.

It is planned to measure the chelate stability constants with metals of the biologically tested compounds and relate these physical measurements with the radiation protection or sensitization demonstrated by the compound.

Significance of Research: Injury due to radiation is becoming a greater hazard as uses of radiation and exposure to radiation become more common. These hazards are found in nuclear warfare, in radiation treatment of cancer, in accidents in nuclear reactors and in space travel, to give some examples. It is best to avoid the radiation, or employ shielding where this is possible. But this is not always possible.

Certain chemicals have been shown to reduce the damage to tissues caused by radiation. A common characteristic of most of the better radiation protection chemicals is their ability to "chelate" or react with metal ions and bind them tightly. If the reasons for this relationship are understood it may be possible to rationally prepare specific compounds that combine the properties of excellent radiation protection with low toxicity and thereby become useful for protection of humans against radiation. At the

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present time no compound can be used clinically because none has been discovered thus far that gives sufficient protection at concentrations in which it is safe for use.

Publications:

Weidner, J. P. and Block, S. S. "Trifluoromethyl Thiolsulfonates and Their Reactions with Mercaptans and Amines." J. Medicinal Chemistry, Vol. 10, November 1967.

RH 00430-03

CELLULAR LOCALIZATION OF XENON BY MICRORADIOGRAPHY

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Research Objectives: It has been shown in our laboratories that xenon gas, in the presence of oxygen, markedly potentiates the effects of X-irradiation. Because xenon is chemically inert, the site and mechanism of its potentiating action are of great importance in radiobiology. Using the methods of microradiography we will attempt to localize xenon at the intracellular level and to quantitate the amount of xenon present at specific sites.

Progress Report: Up until now our research efforts have been directed toward I. studying the effects of xenon gas on the survival of mammalian cells (HeLa, S-3) and II. the development and testing of microradiographic equipment for intracellular localization of xenon.

I. The survival of type S-3 HeLa cells (as measured by clone formation 14 days post-irradiation) has been determined with the cells exposed to the following atmospheres:

1. Air (0.95), CO_2 (0.05)
2. He, O_2(0.20) and CO_2(0.05)
3. Xe, O_2(0.20) and CO_2(0.05)

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The pressures of He and Xe were varied so as to produce total pressures from 1 to 8 atmospheres. Irradiation of the HeLa cells were performed in a pressure vessel that holds four 6 cm plastic petri dishes. A gassing system was designed and assembled that allows for precise delivery of each gas to the pressure vessel. The pressure vessel was held in an exactly reproducible position over the target of a 280 Kv X-ray machine by a support jig that was locked to the body of the machine.

Our observations on the effects of the various atmospheres listed above on the radiation survival of Hela cells may be summarized as follows:

1. Radiation survival under air or He, atmospheres (1) and (2), is the same regardless of pressure.

2. Xenon potentiates the effects radiation and this depends upon the time (degree of saturation) for which the cells are exposed to the xenon atmosphere prior to irradiation. The following table lists the per cent survival for cells exposed to 470 R in air (Atmosphere 1) and xenon (Atmosphere 3) where the air was at 1 atmosphere pressure and the partial pressure of xenon was 3.75 atmospheres. The cells were irradiated 4 hours post plating.

Exposure Time to Xenon	Survival Relative to Air*
4 minutes	27.6%
0.5 hours	14.5%
1.0 hours	16.7%
2.0 hours	13.3%
3.0 hours	8.3%

*5.6% of cells irradiated in air survive a dose of 455 rads.

Calculations of the rate of diffusion of xenon gas through the 2 mm of growth medium that overlies the HeLa cells attached to the bottom of the petri dishes indicate

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that approximately 3 hours are required for 95% saturation. Our cell survival data, and dosimetry data described below, substantiate these calculations.

It can be shown by relatively simple calculations that the amount of energy absorbed in the growth medium per roentgen of 280 Kv X-rays will be 26% greater when the medium is overlayed by an atmosphere composed of Xe (3.75 atmos.), O_2(0.2 atmos.), CO_2(0.05 atmos.) than when it is overlayed by air. Using a LiF thermoluminescent dosimeter having an average particle size of 21 microns, irradiated under conditions identical to the cells, a dose increase of 17% after 3 hours exposure to Xe gas was measured. This somewhat lower value is probably the result of the LiF powder resting upon the bottom of the petri dish where equilibrium between the xenon-containing medium and the powder did not exist. At the present time, the fine grade LiF powder is being used to measure the dose to the HeLa cells under the various conditions of irradiation. On the cellular scale this is still a crude approximation. However, this is the current state of the art.

At this stage of our investigations on the effects of xenon gas on cell survival, we have reached two rather dramatic conclusions:

1. In contrast to earlier reports by Ebert and Howard (1) and Evans (2) that showed xenon to be a protective agent, we find that xenon markedly potentiates the effects of X-irradiation on cell survival.

2. The potentiating effect of xenon may be likened to a dose increase of 58%. The measured dose increase due to the physical presence of xenon gas was 17% and, according to calculations, this may not exceed 26%. We see, therefore, that by mechanisms yet to be described, the presence of an inert gas at or within the HeLa cell at the time of irradiation will potentiate the effects of the radiation in proportion to a dose increase of 25%. For HeLa cells, a dose increase of 25% will reduce cell survival by a factor of one third.

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II. The microradiographic equipment built five years ago has been completely reconstructed and its operation is being tested at this time. This microradiographic equipment is designed to operate in the range of 500 to 20,000 volts with tube currents as high as 40 milliamperes. Monochromatic X-rays from a secondary radiator assembly will be routinely used for specific element localization.

Significance of Research: The anesthetic effects of the rare gases have been recognized for over twenty years. That these same gases are able to modify radiation survival has been known for over ten years, however, this aspect of their biological action has received only scant attention. Previous reports have shown that xenon protects the broad bean root (as measured by growth rate) and mice (as measured by survival) from the effects of X-irradiation. Although we initiated our investigations expecting to find that xenon would protect cells in culture, our findings to date indicate marked potentiation. We do not as yet have an explanation of these conflicting results but our continued research using other types of cells. and the localization of xenon within these cells, should vastly clarify our understanding of where radiation acts upon cells and how the effects of radiation may be modified.

Publications: None

RH 00434-03 RADIATION CHEMICAL STUDIES ON HEMOCYANINS

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Research Objectives: Deprived of oxygen, man dies within minutes. The body, through a coorinated series of mechanical and biochemical steps, takes in oxygen from the air and transforms it into the energy needed for life. Atomic radiations interfere with the vital function of oxygen. Why? To attack this problem, we have focused our investigations on a model protein molecule, hemocyanin, which, like hemoglobin,

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combines with gaseous oxygen in the blood and releases it to the tissues. Atomic radiations prevent the uptake of oxygen by hemocyanin. Our objective is to find out how and where, by systematically examining the process down to the level of the individual atoms making up the giant hemocyanin molecule. With such information we can hope to improve the treatment of cancer where oxygen, in combination with radiation, already plays a significant and often life-saving role.

Progress Report: Hemocyanin is an oxygen-carrying protein like hemoglobin, except that it contains copper atoms instead of iron. Oxygen gas is fixed by combining with the copper atoms which in turn are bound directly to the hemocyanin protein. Solutions of oxygenated hemocyanin are deep blue. When the oxygen is removed the protein becomes nearly colorless. The hemocyanin is obtained from the blood of marine animals, such as crabs and snails, and used by us in its natural state, that is, in its own serum.

We have established that irradiation of oxygenated hemocyanin with gamma or X-rays produces no apparent effect as measured by the oxygen capacity of the protein before and after irradiation. This result seemed surprising, but it turned out that the effects of radiation were manifested if the oxygen was removed from the protein after irradiation. Subsequently, upon reoxygenation, we found that the irradiated hemocyanin took up less oxygen. In fact, the higher the radiation dose, the greater was the loss in oxygen-carrying capacity.

We traced the radiation-induced loss of oxygen-carrying to hydrogen peroxide which is formed in oxygenated solutions during irradiation. The hydrogen peroxide cannot attack the oxygenated protein, but as soon as the oxygen is removed, the hemocyanin is attacked by the hydrogen peroxide. We proved this to be the case by adding hydrogen peroxide to non-irradiated hemocyanin and found that the effects were identical to those found by a dose of radiation which produced an equivalent amount of hydrogen peroxide.

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How does radiation via hydrogen peroxide attack hemocyanin? What happens is the transformation by hydrogen peroxide of the protein-bound copper atoms. The transformation of the copper atoms by hydrogen peroxide takes place by the process of oxidation in which the copper atoms give up electrons. Consequently, the "oxidized" copper atoms, all of which are now in the so-called cupric state, are no longer able to accept electrons from oxygen molecules to form chemical links-hence oxygen uptake by hemocyanin is prevented.

The attack by hydrogen peroxide is prevented when hemocyanin is combined with oxygen, hence no radiation effect is observed when oxygenated hemocyanin is irradiated and maintained in the oxygenated form. In oxygen-free hemocyanin, however, the copper atoms are in the so-called cuprous state and become susceptible to attack by hydrogen peroxide.

We encountered a surprising phenomenon with a hemocyanin obtained from a large snail-like marine animal known as a whelk. With increasing doses of radiation the oxygen-carrying capacity was destroyed in the usual manner. However, as the radiation doses continued to increase, a point was reached at which no further loss of oxygen-carrying capacity occurred. Instead, as the dose of radiation increased, the hemocyanin regained its ability to carry oxygen!

The regenerative action of high doses of radiation was traced again to hydrogen peroxide. It turns out that as the radiation dose increases, the amount of hydrogen peroxide formed increases to the point where its concentration exceeds the concentration of the oxygen reacting copper atoms. At this point, the dual nature of hydrogen peroxide becomes evident. That is, it acts as a reducing agent, instead of an oxidizing agent, and converts the cupric copper to the cuprous form, and thus regains the ability to once again combine with oxygen.

Hemocyanin is more than just an oxygen-carrier since functionally it seems to represent a primitive or primeval form of molecule which eventually evolved into

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the more specialized and more efficient forms of proteins known as enzymes--the catalysts of the body. For example, besides oxygen-carrying, hemocyanin can break down hydrogen peroxide and convert organic molecules such as alcohol and amines into other forms. All of these functions are somehow related to the way the copper atoms are attached to the hemocyanin molecule.

Hemocyanin, being a protein, is composed of thousands of amino acids which link up to form the giant protein molecule. Somewhere in this enormous molecular structure, the copper atoms are attached to one or more amino acids in such a way that enables this metal-amino acid site to assume all of the previously mentioned enzymatic-like functions.

We have studied the catalytic properties of simple model compounds formed between copper and individual amino acids. From these investigations we have been able to deduce the probable atoms to which copper is attached in hemocyanins. We have found that copper-amino acid compounds--called chelates--are capable of efficiently breaking down hydrogen peroxide only when certain unique conditions are met. To appreciate this point it should be mentioned that the copper atom normally has four positions or points available with which it can attach itself to other atoms. Of these four positions, the catalytically active copper must have two, and only two, of these positions occupied by nitrogen atoms which can be furnished by simple amino acids or amino acids in the hemocyanin protein molecule. The two other positions must be available to the molecule being transformed or fixed, such as hydrogen peroxide. If one of the nitrogen atoms is replaced by an oxygen atom, also available from amino acids, the catalytic properties of the copper decrease drastically. It is possible that differences in quantitative behavior of hemocyanins from different animal species may reflect the fact that in one species the copper is attached to hemocyanin by two nitrogen atoms and in another by one nitrogen and one oxygen atom.

In fact, we have identified the two amino acids which, either alone or in combination with another amino acid, are most probably attached to copper in the hemocyanin molecule. These amino acids are histidine and arginine. Inasmuch as copper is a

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component of many enzymes necessary for life, it is obvious that our findings open up new areas for research far beyond the apparently esoteric problem of how radiation destroys the oxygen-carrying capacity of a protein obtained from crabs, snails, and lobsters.

Significance of Research: Atomic radiations kill or injure by interfering with a variety of biochemical processes needed to sustain life. We are investigating a primitive copper-containing molecule, hemocyanin, which by itself is capable of performing biochemical reactions normally carried out in the cell by a large number of specialized protein molecules--the enzymes or biological catalysts.

When hemocyanin is irradiated with relatively small doses of radiation, it loses one of its properties, namely the ability to combine with oxygen. Our research has answered the question as to how radiation affects oxygen-carrying and has also thrown light on the way in which hemocyanin must link with copper so as to have catalytic functions. With such knowledge we can undertake more sophisticated investigations to modify the actions of radiation on living tissue. For example, we can combine radiation treatment of cancerous tissues with drugs deliberately chosen to enhance the action of radiation. Similarly, we can choose drugs which can protect those vital biochemical processes which we find are susceptible to radiation damage.

In summary, then, our research can help us enhance or diminish the actions of radiation on man in health and disease by revealing which molecular processes and structures in the cell are modified by radiation.

Publications:

Schubert, J. and White, E. R. "Radiation Chemical Studies on Hemocyanins." Abstract Ge-3, Radiation Research, Vol. 31, No. 3, July 1967.

Schubert, J., White, E. R. and Becker, L. F. "Radiation Chemical Studies on Oxygen-Carrying Proteins, Hemocyanin." In Advances in Chemistry Series, Vol. 81, Part I, Robert F. Gould, Editor, American Chemical Society, Washington, D. C. (1968), Chapter 34, pp. 480-495.

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00455-03	COMPARATIVE EFFECTS OF TWO RADIATION EXPOSURE REGIMES	DONALD G. WILLHOIT, SC.D. DEPT. OF ENVIRONMENTAL SCIENCES & ENGINEERING UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NORTH CAROLINA

Research Objectives: The purpose of this research is to study the survival times of mice exposed daily to gamma radiation. One series of animals is receiving continuous exposure (23½ hours per day) and a second series of animals is receiving identical daily exposures, but administered in a period of 30 minutes. Blood changes are also being studied to determine if they may be used as a measure of radiation injury.

Progress Report: Survival times of LAF_1 mice have been determined for daily exposures of 75, 150, 225, 300 and 600 R per day. In every instance the mean survival time of the animals in the fractionated exposure group was less than for the corresponding continuous exposure group.

Detailed quantitative evaluation of the data has not been attempted as yet since, due to the experimental design, completion of all of the experiments is necessary before this may be done. A plot of survival-times of mice versus total accumulated dose shows a decrease in the total dose required to produce death as the dose rate is reduced. There are two peaks in this generally decreasing dose-survival-time relationship where the trend is temporarily reversed. These occur at time periods which are characteristic of death due to effects on white blood cells and red blood cells, respectively. We have observed that these peaks are much more pronounced in the group receiving continuous radiation exposure compared with the group receiving the daily fractionated exposure. Evaluation of the hematologic data is now in progress to determine if this finding is reflected in the blood change patterns also. The experiments are progressing on schedule.

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Significance of Research: It is explicitly stated that the rationale underlying the establishment of radiation protection guides for occupational and population exposures is that the biological effects of radiation are dependent on the total accumulated dose irrespective of dose-rate or method of dose protraction. The results of this research thus far suggest that short daily doses produce greater biological damage than the same daily dose in a 24 hour period.

Publications: None

(1) RH 00474-01 RADIATION AND PROTECTION IN ENZYME SYSTEMS

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Research Objectives: Several hypotheses have been proposed during the last few decades for explaining the basic molecular mechanism of radiation effects and of protection. One of these is the charge transfer complex hypothesis, which is the basis for all of our investigations. Accordingly, we are investigating quite a few substances (protective and non-protective) by means of optical absorption and electron spin resonance studies in order to determine whether or not these substances form a complex (and if so, what type of complex) with metal ions. In this way, it could be shown that the formation of a complex is not sufficient enough to give a protective effect but that a charge transfer complex has to be formed. In the latter case, an electron is transferred to the substance under investigation, e.g. a metallo-enzyme restoring the original electronic configuration present before irradiation.

Progress Report: Transition metal ions, e.g. Fe^{3+}, Cu^{2+}, etc., play a very important role in life. They are contained in many of the macromolecules which regulate the different biological functions. Since the amount of these metal ions in these macromolecules is very small (tracer amounts), it used to be very difficult to detect them. Thus, only during the last few decades it was found that the enzyme catalase,

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which decomposes hydrogen perox de, contains the metal iron. Investigations done during the last few years have demonstrated that other biologically important macromolecules contain metal ions, which are part of their active site (group). The most recent experiments done in several laboratories across the world suggest that even the enzyme RNase might contain copper in its active site.

These metals are usually present in an ionized form. In the case of catalase it seems to be Fe^{2+} and Fe^{3+}. The interchange (transition of an electron) between these two ionized forms seems to be responsible for maintaining the biological function, in this case, of an enzyme. One might picture such an interchange as

(a) $$Fe^{2+} \rightleftharpoons Fe^{3+}$$

and a qualitative description of it is given by a redox potential. In order to maintain an optimum biological activity, this redox potential has to be kept reversible. Ionizing radiation will, of course, shift this equilibrium to Fe^{3+} due to (1) a direct process or (2) by an indirect effect produced by the oxidizing substances, such as OH and O_2^-. A similar effect (radiomimetic substance) can be produced by oxygen. High radiation doses or high oxygen tension results in a shift to Fe^{3+}. Under these conditions, the power (amount) of reducing substances, such as cysteine, etc., which under normal conditions would be able to restore the equilibrium of (a), is completely abolished. In this connection it might be of interest to note that a prolonged inhalation of pure oxygen will result in a biological damage. For this reason it was pointed out that our astronauts can inhale pure oxygen no longer than 2 weeks. And, as is known from reports by NASA, even a shorter period of time has already caused certain side effects, such as on bone, etc.

The goal of our investigation is, therefore, to investigate whether or not the radioprotective, the non-protective, and the radiosensitizing substances form a complex with the metal ions present in the macromolecule. A more detailed investigation should clarify, then, the type of complex formed. Since oxygen, as mentioned above,

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influences the redox-potential as well as the indirect radiation effect, the mechanism of the oxygen effect, which is still unknown, is under investigation, too. Moreover, since the redox-potential depends on the pH value, the investigations are also done at different pH values. The methods we use for investigating these complexes are: electric spin resonance and optical absorption studies.

The results obtained thus far can be summarized as follows: substances, such as glucose, etc. which do not form any type of complex with the substance under investigation, modify the radiation effect only slightly. They act as a free radical scavenger, when they are added to an aqueous solution of e.g. catalase. This led us to conclude that complex forming substances give the best radioprotective effect. When, however, the non-protective amino acids, such as serine, alanine, etc. were investigated, we found that they form a complex with metal ions; however, they do not act as an electron donor or reducing substance. On the contrary, sulfhydryl containing amino acids, such as cysteine, reduced glutathione, cysteamine, etc. form a charge transfer complex with the metal ions, and thus restore the original valency of the metal ion which had been changed under the influence of radiation to a higher valency. The association constants of these charge transfer complexes seem to be quite different from the ones obtained for metal ion-non-sulfhydryl amino acid complexes. The latter ones are stable, that is, they exhibit the same spectra even after a few days. The charge transfer spectra of these exhibit the original (e.g., Cu^{2+}) spectrum when they are stored (at room temperature or in a refrigerator) under normal conditions. This re-oxidation cannot be observed when they are stored in a nitrogen atmosphere; while bubbling O_2 through the solution for only about 5 min. results in the original Cu^{2+} spectrum. It is still unknown whether a chelate is formed in the case of the charge transfer complexes and, especially, whether or not the SH groups are involved in the binding. As Benesch and Benesch have shown, there is only a small percentage of the SH groups ionized at neutral pH, a pH value, where most of our previous experiments have been done.

The results obtained thus far support the working hypothesis described above.

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Significance of Research: With the results obtained, we hope to be able to demonstrate the mechanism of action of radioprotective and radiosensitizing substances. In addition, since most of the substances are used as drugs in medicine, it is hoped we will be able to elucidate the mechanism of action of certain drugs. To a certain extent, this might also contribute to the question of toxicity of certain drugs. Thus, we have just completed a study about the effect of penicillamine, where the d-isomer is very important for curing certain diseases while the l-isomer is toxic.

To have a reasonably adequate understanding of the situation, we should know (1) how many groups can be bound; (2) how strongly they are bound; (3) the nature of the various combining sites; (4) their locations in the structure of a macromolecule, e.g. enzyme; and (5) the biological significance of it all. That is, one wishes to answer the question put by Scatchard (1949) and by Scatchard et. al. (1954); "How many? Where? Why? What of it?"

Publications:

Lohmann, W., Momeni, M. and Nette, P. "On the Possible Involvement of Charge Transfer Complexes (Redox Systems) in Radioprotection." Strahlentherapie Band 134, Heft 4, 1967.

Lohmann, W., Evans, T. C. and Momeni, M. "Paramagnetic Resonance and Optical Absorption Spectra of Different Amino Acids in Aqueous Solutions of Copper." Abstract Ae-7, Radiation Research, Vol. 31, No. 3, July 1967.

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RH 00479-02	HEMATOLOGIC RESPONSES TO NON-HOMOGENEOUS IRRADIATION	DONALD G. WILLHOIT, SC.D. DEPT. OF ENVIRONMENTAL SCIENCES & ENGINEERING UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NORTH CAROLINA

Research Objectives: The objectives of this research are to:

1. determine if changes in the peripheral blood following partial body exposure may be used as indicators of lethal radiation injury, and

2. develop expressions for dose and dose distribution factors for non-uniform radiation exposure.

Progress Report: Hematologic studies have been completed for groups of rats following whole-body exposures of 435, 700, 960, and 1300 R; upper body exposures of 520 and 1300 R; and lower body exposures of 700, 870, and 1220 R. For each of the three exposure conditions studied thus far, there is an immediate drop in the lymphocyte count which is related to the magnitude of the dose. The decrease in the lymphocyte count is greatest under whole-body exposure conditions and least under upper body irradiation conditions. This is thought to be due to the relative amounts of lymphocyte-producing tissues irradiated. There is a corresponding increase in the neutrophil count 3 to 6 days after exposure in each of the three groups studied and also appears to be related to dose but in a different manner than the decrease in lymphocyte count. An increase in the hematocrit values was observed in the lower-body high-dose group which reflects fluid balance disturbances due to injury to the gastro-intestinal tract. An increase in the hematocrit values was also observed in the upper-body high-dose group but occurred approximately 6 to 9 days after exposure. Sufficient data have not been collected as yet to construct dose-effect curves or to develop dose distribution factors. The experiments are proceeding on schedule.

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Significance of Research: Biological effects of radiation are greatly influenced by the distribution of dose within the irradiated organism. Precise quantitative comparisons of data from experimental animals with data from humans accidentally exposed are not possible due to the non-uniform exposure conditions of the humans, and lack of animal data for dose distributions corresponding to those encountered in the human exposures. The purpose of this study is to provide quantitative dose-hematologic response relationships for non-uniform dose distributions in X-irradiated rats.

Publications: None

RH 00483-02 ALTERATION OF GENETIC REPLICATION

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Research Objectives: In antecedent studies both penicillin and actinomycin D exhibited a protective effect when lethals were determined following irradiation. Since both of these agents are effective in dosages therapeutically useful in mammals without toxic effects, it was thought desirable to see whether antibiotic inhibits the production of lethals by irradiation in mammals as well as in Drosophila. In order to make the study more informative, it was decided to utilize actinomycin D and puromycin whose effects on protein synthesis are known. Therefore, mice have been treated with various dosages of antibiotic before and after irradiation and determining the number of dominant lethals in subsequent periods. Males were irradiated, mated, early deaths and implantations in females impregnated noted, and the number of dominant mutants calculated.

Progress Report: Spermatozoa are highly sensitive to induced lethal mutants immediately after irradiation. This sensitivity then declines, and there is reduced

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sensitivity during the second week with the highest sensitivity reached during the third week after irradiation. Spermatids at the time of irradiation have reached a stage of development for fertilization by the third week. From the fourth through the thirteenth week is a relatively sterile period, following which there is a return to fertility. In view of these facts, attention has been concentrated on the possible effects of actinomycin D during the first three weeks, represented in Table 1. The amount of actinomycin D given and the times of administration before and after irradiation are varied. Throughout the studies, irradiation was given as a single treatment of 175 roentgens (400 kv). The dominant lethal mutation rate is computed as the number of early deaths divided by the number of implantations from which the early deaths have been subtracted. When actinomycin D was given before irradiation, or before and after irradiation, there is an increase in dominant lethal mutations, whereas there is a decrease in those groups with actinomycin D was given before irradiation, or before and after irradiation, there is an increase in dominant lethal mutations, whereas there is a decrease in those groups with actinomycin D administered only following irradiation. With the exception of experiment 11, these data represent the results of matings within the first two weeks divided into matings during days one through four, five through nine, and ten through fourteen, with slight variation. In experiment 11, the tests were done so that the matings were carried out on days one through four, five through fourteen, and fifteen through nineteen post-irradiation. Review of results for respective intervals failed to show any consistent pattern among them that would suggest a trend in the influence of actinomycin D on the number of dominant lethals following irradiation when data for intervals are compared. The study will be concluded by adding data for the sterile period and by determining whether prolonged administration of actinomycin D has any different effect than those reported. The differences in rates are not great, and the grant is permitting a careful and extensive study to determine whether the antibiotic studied has any useful properties in altering the mutation rate following irradiation.

Significance of Research: Both the therapeutic and mutagenic effects of irradiation may be altered by changing the environment. The study in progress hopefully

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will give some indication about whether antibiotics of the type utilized may be useful in either protecting against the production of lethal mutations by irradiation or in altering the duration of various stages of spermatogenesis and thereby changing relative sensitivity to mutants induced by irradiation, since the various stages are known to have different sensitivities to this effect of irradiation.

MUTATION RATE OF DOMINANT LETHALS

				175 R				Control			
		Hours		No Antibiotic		Actinomycin D		No Antibiotic		Actinomycin D	
Exp.	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)	(K)
1	.520	12		839	.254	253	.297	706	.066	283	.084
2	.264	24	24	654	.176	1200	.209	475	.055	855	.048
3	.264	2	24	3047	.185	2288	.195	1215	.055	2098	.067
4	.264	24	2	341	.209	288	.305	151	.034	232	.079
5	.264	24,2	2	1091	.114	1089	.112	583	.069	355	.066
6	.264	24,2	2	4635	.224	6325	.246	2757	.077	4247	.062
7	.066		2	2653	.422	3283	.406	1474	.073	3347	.071
8	.125		2	419	.225	501	.168	462	.064	299	.035
9	.132		2	3042	.239	3401	.235	1651	.062	2258	.078
10	.263		2	1090	.223	1091	.151	1424	.049	1071	.053
11	.263		12	689	.264	705	.257	802	.091	464	.047

(A) ug Actinomycin D per Gram
(B) Pre-irradiation inoculation
(C) Post-irradiation inoculation
(D) Implantations
(E) Lethals
(F) Implantations
(G) Lethals
(H) Implantations
(I) Lethals
(J) Implantations
(K) Lethals

Publications: None

MECHANISMS OF RADIATION DAMAGE AND EFFECTS MODIFICATION

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(3) RH 00507-01A1	THE VALUE OF RADIOPROTECTIVE COMPOUNDS	RICHARD I. H. WANG, PH.D., M.D. DEPT. OF PHARMACOLOGY MARQUETTE UNIVERSITY SCHOOL OF MEDICINE MILWAUKEE, WISCONSIN

Research Objectives: The primary purpose of the proposed study is to achieve the most desirable method of protecting laboratory animals against the lethal effect of ionizing radiation. To reach this goal the study is subdivided into eight specific aims of investigation. The study will include the pharmacology and toxicology of each promising radioprotective chemical. Enhancement of the protective effect will be investigated by using mixtures of new and existing radioprotective agents. Such an approach has been shown to be effective. Further enhancement of protective action will be investigated by combining pre-radiation administration of chemicals and post-radiation transplantation of bone marrow. Sites of chemical protection will be studied by combining partial body shielding and chemical administration. Repeated exposures to sublethal and supralethal doses of ionizing radiation will provide information on both the bodily recovery processes and on the phenomenon of radioresistance. The biological response of animals exposed to both 300 kVp X-ray and a linear accelerator source will be studied. These studies in animals will hopefully lead to finding effective radioprotective measures which can eventually be applied to man.

Publications: None

(3) RH 00520-01	IN VIVO REPAIR MECHANISM IN IRRADIATED ANIMALS	JACK SCHUBERT, PH.D. DEPT. OF OCCUPATIONAL HEALTH UNIVERSITY OF PITTSBURGH PITTSBURGH, PENNSYLVANIA

Research Objectives: The aim of this proposal is to investigate systematically the mechanisms by which non-lethal doses of cyanide modify the actions of ionizing radiation in mammalian systems. One approach will utilize the kinetic patterns

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RH 00520 (Continued)

of cyanide-induced inhibition and recovery of tissue cytochrome oxidase in vivo and in vitro as a probe with which to explore the time relations and nature of the primary biochemical processes involved in the damaging action of ionizing radiations. The principal investigator has recently found that there exists a relationship between the degree of radioprotection by cyanide and the length of time cytochrome oxidase remains in the inhibited state. It is hoped that the proposed investigations will lead to new and improved chemical means for prophylactic and therapeutic treatment of radiation injury as well as new approaches to the enhancement of tissue radiosensitivity. If a functional relationship between cytochrome oxidase activity and radiation action is established, then it is hoped, in addition, that a new approach to cancer radiotherapy can be developed in which the radiosensitivity can be increased by unprecendented factors of ten or more prior to radiotherapy. Such an approach, involving the use of penicillamine for depleting copper, hence reducing cytochrome oxidase, has already yielded impressive results in the treatment of advanced melanoma in humans. Experimental studies along these lines are planned in order to confirm the validity of this approach.

Publications: None

(1) RH 00528-01 AMINOTHIONIMIDES AS RADIATION PROTECTIVE AGENTS

DONALD T. WITIAK, PH.D.
COLLEGE OF PHARMACY
OHIO STATE UNIVERSITY
COLUMBUS, OHIO

Research Objectives: This research concerns the synthesis and biological evaluation of aminothionimides. Aminothionimides represent molecular structures related to the β-mercaptoethylamines which have been shown to be protective. It is possible that the latter compound works, at least in part, by trapping highly reactive free radicals resulting from homolysis or splitting of water by ionizing radiation. Study of aminothionimides, which on theoretical grounds may be more effective trappers of these free radicals, may aid in the understanding of the mechanism of action of radiation protective agents.

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Progress Report: A most important goal of research with chemical protective agents has been to decrease the effects of ionizing radiation on living systems, particularly human beings. A direct approach to this problem is derivable from studies of radiation effects on the vital components of cells. These may be studied by direct collision with ionizing radiation or by studying the effects of the products of radiolysis of water and oxygen. There is evidence that during the irradiation of aerated water hydroxyl radicals (HO.) and perhydroxyl radicals (HOO.) are formed along with H. and H_2O_2. Further, it is known that almost all irradiated proteins lose their specific biological properties, and changes in their physical properties occur upon irradiation. This change is at least in part due to oxidation of sulfhydro groups on proteins by the radiolysis products of water and oxygen. Support for this proposal may be found in the observation that compounds capable of lowering oxygen tension in tissue are able to exhibit some protection against ionizing radiation. The reason for this observation may be due to the presence of fewer perhydroxyl radicals derivable from a combination of oxygen and hydrogen atoms.

Many series of compounds have been tested for anti-radiation properties and extensive lists are available. Similarly, many investigators have proposed a variety of mechanisms of action for these compounds. While we do not wish to imply that trapping free radicals represents the only mechanism of action of radiation protective agents, we agree with others to the extent that such a mechanism probably represents a very important factor. Inspection of compounds which exhibit protective ability in biological systems indicates that many of these should readily react with perhydroxyl radicals and similar species.

As early as 1955, Alexander and co-workers noted that a common feature of relatively good protective agents was an unshared pair of electrons. This observation was interpreted on the basis of a free radical trapping mechanism. In addition, some of the best protective agents in mammals contain a thiol or -SH group. Compounds containing an amino ($-NH_2$) group as well as a thiol group were the most effective.

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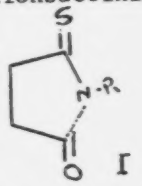
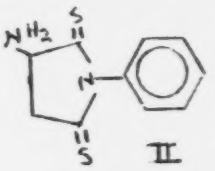
In our laboratories a number of simple monothionsuccinimides were synthesized. Monothionsuccinimides (R=H) at a dose of 4.5×10^{-4} moles /Kg (mice) protected animals (X-ray dose - 700) to the extent that the mortality rate at 30 days was 60%. Introduction of methyl on the imide nitrogen (R= CH_3) reduced the activity to 30% even though a higher dose (15×10^{-4} moles / kg) was employed. Conversely, introduction of phenyl (R=C_6H_5) on the imide nitrogen increased the protection to 60% at only a slightly higher molecular concentration (10×10^{-4} moles /Kg) and utilization of a methoxyphenyl (R=C_6H_5-OCH_3) group increased the activity to 50% at the same molar concentration. At one-half (4.5×10^{-4} moles /Kg) the molar concentration of the monothionsuccinimide of the methoxyphenyl compound exhibited the same activity as the unsubstituted compound.

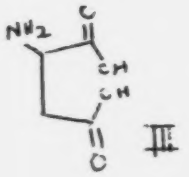


I

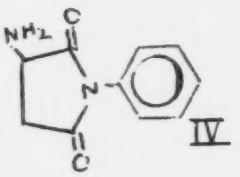
Since one can introduce a phenyl (R = C_6H_5) ring on the imide nitrogen and maintain good biological activity, and since aromatic substitution increases the stability of the C = S groups as well as the stability of the imide ring (Witiak, etal., J. Org. Chem., 30, 3721 (1965)) we have directed our more recent studies toward the synthesis of α-amino-N-phenylthionsuccinimides. Insertion of the amino group (-NH_2) should further enhance the radiation protective activity of the compound and render the molecule a better free radical trapping agent. Synthesis of this compound from aspartic acid involves six steps. Of these we have successfully completed five and have prepared α-amino-N-phenyl-succinimide.



II



III



IV

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Significance of Research: The lack of information which can be found concerning chemical, physical, and biological properties of thionimides emphasizes the need for research in this area. The work proposed will be contributory to an understanding of these properties as well as expand our knowledge in amino acid chemistry. Investigation of the biological properties of aminothionsuccinimides represents an extremely fertile field. Synthesis of these compounds will not only enable one to evaluate their protective ability against ionizing radiation, but will also make these compounds available to cancer screening programs and general biological evaluation. Studies with free radical chemistry should further our understanding of these species in animals.

Publications: None

RH 00529-02

MERCAPTOCYCLOPROPYLAMINE: RADIATION-PROTECTIVE AGENTS

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Research Objectives: A review of the literature suggests chemical modification of the mercaptoethylamine structure continues to be worthwhile in the synthesis of new radioprotective agents; some highly active derivatives have been reported. This project concerns the synthesis and biological evaluation of structurally rigid compounds which represent molecular modifications of mercaptoethylamines I and II.

$H_2NCH_2CH_2SH$ $H_2NCH_2CH_2CH_2SH$

I. II.

These two compounds are among the most potent radioprotective agents and a study of compounds in which the NH_2 and SH groups are cis (i.e. held on the same side of the molecule) or trans (held on opposite sides of the molecule) should be complimentary

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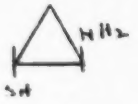
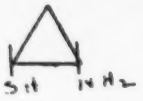
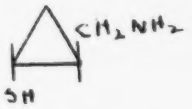
to the work of others concerning in vivo mechanism of action. For preparation and biological evaluation of such cis and trans isomers (compounds with the same formula, but different geometry of substituents) a cyclopropane ring system is desirable. Utilization of the 3-membered ring represents the smallest function for importing rigidity to the molecule and therefore enables synthesis of the cis and trans isomers III and IV or V and VI most closely related to the parent mercaptoalkylamines I and II, respectively.



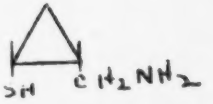
III, trans



IV, cis



V, trans



VI, cis

Progress Report: An extensive literature on the radiation protective action of chemical agents reveals many unrelated compounds exhibit such activity. However, advancement in knowledge of structural requirements for chemical protection against ionizing radiation has continued mainly through modification of structures having known protective effects. While the mechanism of chemical protection still remains obscure, much data has been presented to support a variety of proposals. One of the most important classes of chemical radioprotective agents consists of sulfhydryls or thiol derivatives. Interest in this series was initiated by the discovery of Patt and coworkers that the amino acid cysteine had excellent activity. Subsequent investigations demonstrated that many other thiol (SH) containing compounds including I and II had radiation protective activity but stereochemical studies relative to cis and trans isomers have not been investigated.

Compounds III, IV, V, and VI have never been made although a number of theoretical approaches may be considered for their preparation. For example, addition of diazomethane (CH_2N_2) to double bonds (CH=CH) may afford cyclopropane derivatives and consequently the reaction of CH_2N_2 to $CH_3CS\text{-}CH = CH\text{-}CO_2E+$ was studied. In the

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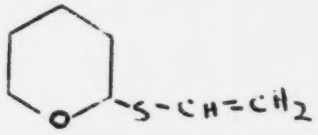
case of this reaction no cyclopropane ring could be detected, but the results were still of significance from a chemical standpoint since the products formed could be studied in terms of stereochemistry of the starting material. Another approach involves reaction of ethyl diazoacetate ($EtOC\overset{O}{\overset{\|}{C}}CH_2N_2$) with compounds containing double bonds. Reaction of vinylthioacetate ($CH_2 = CH\text{-}SA_c$) with ethyl diazoacetate affords a mixture of compounds, but 20% of the product contains compounds with cyclopropane rings from which III, IV, V, and VI may be prepared.

The observations that cyclopropane containing compounds are derivable from $CH_2 = CH\text{-}SA_c$ prompted exploration of the reaction of ethyl diazoacetate with VII with the hope of increasing



VII

VIII

the yields of VIII containing a cyclopropane ring. In fact VIII has been isolated in 40% yield and we are presently studying the reactions involved in converting VIII to III, IV, V, and VI.

Compounds will be studied biologically by Dr. David P. Jacobus and his co-workers, Division of Medicinal Chemistry, Department of the Army, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. We are of the opinion that studies of the isomers III, IV, V, and VI proposed will be of value in differentiating possible modes of action of aminoalkylthiols.

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Significance of Research: Studies with the mercaptocyclopropylamines (III, IV, V, and VI) described in this communication may give rise to very effective radioprotective agents. Correlation of stereochemistry with biological activity will compliment the work of others with regard to mechanism of action of mercaptoalkylamines and may suggest new approaches to the study of such mechanisms.

Contributions to the chemical literature will also be of significance since little is known about cyclopropyl mercaptan chemistry and nothing is reported about the compounds proposed or their synthesis. Synthetic aspects of this research is of basic value to the understanding of stereochemistry and mechanism of the reactions involved as well as in terms of the physical, chemical and biological properties of intermediates.

Synthesis of the desired mercaptocyclopropylamines as well as the intermediates will enable preparation of derivatives for other kinds of pharmacological activity. The fact that the " $-OCH_2CH_2N-$ " function represents the structure of importance found in local anesthetics, antihistaminics, cholinergic agents, and many other biologically active compounds indicates that " $-SCH_2CH_2N-$ " will also be of interest in these areas. Appropriate compounds will be prepared containing the cyclopropane ring and biologically evaluated.

Publications: None

(3) RH 00545-01 — REMOVAL OF AM-241 FROM HUMANS WITH DTPA

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DEPT. OF OCCUPATIONAL HEALTH
UNIVERSITY OF PITTSBURGH
PITTSBURGH, PENNSYLVANIA

Research Objectives: Data on the retention, translocation, and elimination of Pu-239 and Am-241 have been accumulated on several humans who were accidentally exposed to the inhalation of these radionuclides in the course of their employment. Since these radionuclides are potential carcinogens when present in bone and/or the lung, even

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in sub-microgram quantities, these patients have been treated with DTPA to increase excretion of these nuclides. We plan to examine the data accumulated, and to continue further treatments and measurements on certain patients, in order to: (1) determine the most effective schedule of treatments for removal of these toxic agents; (2) develop an understanding and a quantitative theoretical model that can be used to predict appropriate dosages of DTPA and treatment schedules for future cases. Cases recently under treatment are providing unique opportunities for the in vivo measurements of these nuclides in various body compartments, and in excreta, as a function of time.

Publications: None

RH 00548-01

EFFECT OF MICROBIAL ANTIGENS OF IRRADIATED MICE

ALICE M. SAVAGE, PH.D.
DEPT. OF ANATOMY
PENNSYLVANIA STATE UNIVERSITY
UNIVERSITY PARK, PENNSYLVANIA

Research Objectives: To study the effects of a lethal dose of X-irradiation on mice and the alleviation of the radiation damage by treatment with bacterial antigens. The treatment allows the animals to live by combating infection and by promoting blood cell recovery. This investigation concentrates on cellular activities important to these two essential aspects of recovery from radiation damage.

Progress Report: When mice are irradiated, their blood cells die and those cells in the body which normally destroy harmful bacteria (phagocytes) are seriously damaged. If left untreated after receiving a lethal dose of irradiation, mice will die from anemia and bacterial infection. The following studies were made to test a possible treatment for this radiation disease: the administration of a substance (a bacterial antigen) which stimulates both the new production of blood cells and the recovery of the damaged phagocytes.

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The project involved: (1) examination of normal control mice, (2) studies on the cause of death of mice given lethal doses of irradiation and then left untreated, and (3) studies of the effect of bacterial antigens on mouse recovery from lethal doses of radiation. The study has focused upon two major aspects of this problem: (1) blood cell recovery, and (2) defense against infection. Each of these will be discussed below.

(1) Blood cell recovery - Presently the transformation of primative cells into white blood cells is being explored and this hits at the heart of a basic biological problem: how is a less specialized cell transformed into one that is highly specialized for a limited number of functions? Regenerating splenic tissue has been obtained and processed for study with an electron microscope and data on the internal cellular changes are now being collected.

(2) Defense against infection - One of the major defense mechanism against bacterial infection consists of a group of cells (phagocytes) distributed throughout the body. These cells are capable of engulfing and destroying bacteria. In irradiated animals, this ability to destroy bacteria is severely impaired and the animals die with a massive infection. Treatment of mice with bacterial antigens prevents infection from developing. Several studies are in progress investigating the effect of bacterial antigens on the ability of phagocytes to engulf and destroy bacteria. Experimental data indicates that the number of phagocytic cells remains the same whether or not the animals were given the antigen. We are now investigating the ability of the cells to destroy bacteria.

Significance of Research: This investigation is a study of the causes of radiation death and the prevention of death by treating animals with bacterial antigens. In a time when there is radioactive contamination in our environment, the threat of radiation damage is always present. It is of the utmost importance to know the harmful effects of this radiation and ways to stimulate recovery if radiation injury

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RH 00548 (Continued)	does occur. The need for research in this area is pressing. The increasingly extensive use of atomic radiation as a source of power increases the chance for radioactive accidents which could affect large numbers of our population. Effective treatment procedures should be available in case such accidents occur. Publications: None	

STUDIES IN BASIC RADIATION PHYSICS AND CHEMISTRY

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00008-08	STUDIES OF RADIATION DAMAGE TO BIOLOGICAL MATERIALS	ROY S. ANDERSON, PH.D. DEPT. OF PHYSICS CLARK UNIVERSITY WORCESTER, MASSACHUSETTS

Research Objectives: Molecules which have been damaged by high energy radiation are generally weakly magnetic. This magnetism is detectable by a highly sensitive radio spectroscopic technique known as Electron Spin Resonance. In addition to detecting the presence of magnetic, radiation-damaged molecules, Electron Spin Resonance spectroscopy can also reveal details such as the location within the molecule at which radiation damage occurred. Single crystals of biological materials are grown and exposed to ultraviolet, X-, gamma-, and neutron radiation. The small concentrations of damaged molecules (between 0.1 and 5%), which are stably trapped in the crystal lattice, are investigated by Electron Spin Resonance spectroscopy.

Progress Report: Research on radiation damage is approached either by the study of the aggregate effects of radiation or by the study of fundamental effects. Aggregate studies are conducted on whole organisms which have been subjected to radiation and which are then examined for overall effects. This approach does not reveal details of how the damage takes place. In contrast, the fundamental studies consider systems at the molecular level and provide information on the specific ways by which radiation alters the constituent molecules of organisms. With the fundamental approach, it is difficult to infer the final effects of radiation damage to complex, whole organisms. However, it is essential that there be acquired specific knowledge on the effects of radiation on simple molecular systems before the effects of radiation on complex organisms can be understood.

In this research the fundamental approach is used. The specific aim is to study the effects of high energy radiation on compounds of biological interest, or of related compounds. Of approximately 200 compounds which have been subjected to damaging radiation, Electron Spin Resonance spectroscopy has shown that approximately 75%

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show that radiation damage has occurred. Complete studies of damaged molecules require that samples be prepared as single crystals. Thus far, it has been possible to grow single crystals of about one-third of those materials which show radiation damage.

Through the use of Electron Spin Resonance spectroscopy, it has been possible to make specific identification of the molecular fragment (radical) which remains after the molecule is damaged by the radiation. Our experiments have shown that radiation damage to organic materials usually results from the breaking of the chemical bonds between the constituent atoms of the molecules. Our results indicate that when crystals are subjected to 10^7 R doses of radiation, concentrations of damaged molecules range from one in a thousand to one in a million. Further, there is a distinct ordering in the radiation sensitivity of the various chemical bonds. In particular, carbon-sulphur bonds appear to be the most sensitive to radiation damage, followed by carbon-nitrogen bonds, carbon-hydrogen, and carbon-oxygen bonds, in descending order.

By examining crystals whose internal structure is known from X-ray diffraction studies, it is possible to determine the precise physical structure of the damaged molecule.

While gamma-radiation has been used most extensively as the damaging agent, X-rays, ultraviolet rays, and neutrons have also been demonstrated to be effective agents. As expected, gamma- and X-radiation produces substantially the same effects. Neutron irradiation, while effective in producing damage, usually produces simpler damage. Ultraviolet radiation has been found to effect only a few, special classes of compounds. Also, certain materials appear to be damaged by hydrogen addition; that is, a free hydrogen atom released from some other molecule attaches itself to a molecule, producing a relatively stable type of damage. This result, first discovered as an effect of gamma-radiation, is now being studied directly by exposing molecules to a stream of hydrogen atoms.

Of interest is the development of an instrument to synthesize Electron Spin Resonance spectra. These spectra are frequently complex and difficult to analyze. To assist

STUDIES IN BASIC RADIATION PHYSICS AND CHEMISTRY

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and to speed the process of analysis, an analog computer has been designed and constructed to simulate observed spectra. This instrument has speeded the analysis process by at least 100 times over previously employed methods.

Significance of Research: Before the question of radiation damage in living systems may be answered, it is first necessary to obtain information on the precise effects of radiation on the molecules of which living systems are composed. This research is yielding information of such fundamental nature. This research will also indicate the tolerance of molecular species to damaging radiation. From this it should then be possible to infer the ways in which radiation affects the functioning of living systems and to determine the permissable levels of exposure to radiation. Through studies of the decay of radical species the ability of living systems to recover from radiation damage may be determined. Finally, from these experiments specific protective agents may be identified which can guard against or assist in recovery from radiation damage.

Publications:

Shrivastava, K. N. and Anderson, R. S. "Electron Spin Resonance Study of γ-Irradiated Single Crystals of Semicarbazide Hydrochloride." Journal of Chemical Physics, Vol. 48, No. 10, May 15, 1968.

RH 00099-07 RBE OF MONOENERGETIC NEUTRONS

HARALD H. ROSSI, PH.D.
DEPT. OF RADIOLOGY
COLUMBIA UNIVERSITY
NEW YORK, NEW YORK

Research Objectives: The immediate research objective is an evaluation of the relative biological effectiveness (RBE) of neutrons as a function of the energy with close correlation between the physical pattern of energy deposition and the resultant biological effect. A resultant long term objective is an inquiry into the basic modes of action of radiation on the cell with particular emphasis on the effects of irradiation at low levels.

STUDIES IN BASIC RADIATION PHYSICS AND CHEMISTRY

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RH 00099
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Progress Report: It is essential in this research that physical characteristics of radiation be precisely evaluated not only with respect to absorbed dose, which is the macroscopic distribution of energy in irradiated tissues but also with respect to microscopic parameters. As a result two kinds of techniques have been developed. In those relating to absorbed dose measurement, tissue equivalent ionization chambers have been employed to determine the absorbed dose in suitably shaped phantoms in specific organ locations (such as the eye, testes, etc.). In order to determine microscopic patterns of energy deposition, instrumentation has been developed to measure energy deposition by individual particles in regions which are as small and smaller than the cell. Spheres of diameters down to 0.1 microns have been simulated in this work.

The biological systems investigated have included the following: thymus, spleen, spermatogonia, and the lens of the eye of the mouse; the mouse embryo and chromosome breaks in maize (the YG_2 system); and Drosophila (genetic effects).

In summary, the principal findings to date are as follows: Inactivation of all cells investigated occurs through the agency of a single neutron particle for energies between approximately 0.5 and 2 MeV. At higher energies (14 MeV) inactivation can be either by one or more than one particle depending on the biological system exposed. With the possible exception of certain types of mutations in Drosophila, inactivation by X and gamma rays is invariably due to the action of more than one secondary particle (electron). However, even when neutron inactivation is by single secondaries, the biological effectiveness varies with energy presumably because of differences of the LET of these secondaries. It has been found possible to account for such changes in a quantitative way in the case of chromosome breaks in corn where a sensitive region and the energy requirements for inactivation may have been identified.

As a result of these basic relations, the biological effectiveness does not only depend on neutron energy but more importantly the RBE of neutrons, relative to X-rays, rises steadily as the dose is reduced. Exceedingly high RBE values have been found which can range up to 100 (and even higher) in the case of opacification of the mouse

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lens and chromosome breaks in Zea mays. The observation of the slight lens opacifications involved has required the development of refined classifications of injury and computer assisted evaluation of data in which the incidence of effects is fitted to a log-normal time dependence.

As a result of the comparison of effects in irradiated animals and in control animals basic relations between radiation effects and aging were demonstrated.

Significance of Research: These findings are of significance in the areas of theoretical radiobiology and of radiation protection. In the former they permit a check on existing theories of the action of ionizing radiation and the formulation of alternate explanations. In the area of human radiation protection the evident absence of any dose threshold for cellular inactivation by low energy neutrons has important implications with respect to the exposure of both radiation workers and the general public.

Publications:

Bateman, J. L. and Bond, V. P. "Lens Opacification in Mice Exposed to Fast Neutrons." Radiation Research, Supplement 7, 239-249, 1967.

Bateman, J. L., Bond, V. P. and Rossi, H. H. "Lens Opacity-Induction as a Function of Environmental Oxygen Concentration During X-Irradiation." Abstract Gc-4, Radiation Research, Vol. 31, No. 3, July 1967.

Johnson, H. A. and Bateman, J. L. "Pathogenesis of the Radiation Cataract in the Mouse." Abstract Gc-1, Radiation Research, Vol. 31, No. 3, July 1967.

STUDIES IN BASIC RADIATION PHYSICS AND CHEMISTRY

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
(1) RH 00206-05	RADIATION EFFECTS IN HETEROGENEOUS SYSTEMS	FRANCIS J. JOHNSTON, PH.D. DEPT. OF CHEMISTRY UNIVERSITY OF GEORGIA ATHENS, GEORGIA

Research Objectives: This research project involves studies of surface and interfacial effects in radiation-induced processes. The objectives are: 1) To determine the modifications to current radiolysis models which are required to explain behavior in heterogeneous systems. 2) To study reaction kinetics in irradiated colloids and 3) To attempt to correlate results in these systems with possible mechanisms of damage and protection in irradiated biological systems.

Progress Report: Other workers have found that collodial sulfur is an effective radioprotective agent in biological and pseudo-biological systems. We have chemically produced colloidal sulfur with a narrow particle size distribution and studied its behavior upon irradiation. The sulfur reacts chemically when aqueous suspensions are subjected to Co-60 irradiation with the formation of sulfuric acid. The unbalanced reaction is

$$S + H_2O \xrightarrow{\sim\sim} H_2SO_4.$$

Particle size in the sulfur suspensions is followed by light scattering techniques. The sulfur colloids used in our work contain molecular sulfur (S_8) in concentrations of from 20×10^{-6} M to approximately 10^{-6}M. The range of particle radii which can be studied by this technique is from 0.45 u to 0.15 u. We have found a markedly greater reactivity for small particles than large. Sulfur particles of radius 0.18 and at a particle concentration of 10^6 cc^{-1} react with a G value of approximately 0.6. The radioprotective effect of sulfur seems to be a result of its acting (at higher concentrations) as an effective scavenger for radiation-produced intermediates and its ultimate conversion to innocuous sulfuric acid. The reaction of the colloidal material is apparently initiated by a radical attack on the surface. The reactive species is evidently the OH radical or precursor to it.

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Colloidal silver halides in aqueous suspension are stable toward ionizing radiation. In the presence of small concentrations of organic additives (alcohol, acetone or acetic acid) colloidal silver is produced upon irradiation. We are attempting to determine whether this is a result of altered interfacial properties or a secondary radical attack.

Significance of Research: This research program will, hopefully, determine the role played by the absorbed ionic layer in radiation effects at interfaces. We hope also to explain an apparent kinetic difference between reactions of radiation-produced intermediates at surfaces and corresponding reactions of normal molecules at the same surface. An understanding of such processes would be most important in explaining and predicting protection in biological systems.

Publications:

Baxter, J. N. and Johnston, F. J. "Gamma Radiolysis Studies of the Chloroacetic Acids." Radiation Research 33, 311-324, 1968.

RH 00253-05

MEASUREMENT OF NEUTRON SPECTRA IN BIOLOGICAL SHIELDS

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Research Objectives: The objective is to make direct measurements of the penetration of fast reactor neutrons through solid shields such as water, graphite and steel for biological protection. The fast neutrons are measured from 2.0 to 20.0 Mev as a function of energy, spatial position, and scattering angle. The measured data are compared with spectra computed by various neutron transport codes as a verification of both the code method and neutron cross section input.

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RH 00253
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Progress Report: A portable fast neutron spectrometer has been developed using the proton recoil telescope principle, with thick silicon surface barrier solid state detectors. The spectrometer must be simple and rugged for submersion in the reactor pool, bathed in a reactor gamma field up to 10^7 rads/hr. It has been extensively calibrated at a 14.0 Mev neutron accelerator. Typical energy resolution is 10 per cent (FWHM) at all energies out to 20.0 Mev, and detector efficiency of 10^{-8}. Linearity in energy response is within 1 percent between 2.0 and 18.0 Mev. This spectrometer is believed to be a significant improvement over the older gas proportional counter telescope, has demonstrated accurate linearity, and durability in the difficult region near reactor cores.

Energy differential neutron spectra have been measured, as a function of penetration distance, in water and graphite. Multi-group transport calculations have been performed for comparison. These show good agreement in general, but require, apparently, improvement in some neutron scattering cross sections.

As part of the biological shield penetration survey, the reactor source leakage spectrum has been carefully examined. At energies above 6.0 Mev, this spectrum is an accurate measure of the true U-235 fission spectrum. As a result, the U-235 fission spectrum has been determined out to 20.0 Mev, with an accuracy considerably better than heretofore published. The data show that the semi-empirical fission spectrum, accurately known at low energies, can indeed be used up to 20.0 Mev.

Significance of Research: The accurate prediction of biological and material dose rate due to fast neutrons in a reactor environment is a primary need in reactor shielding design. The computer transport codes needed for such predictions must provide neutron flux values as functions, simultaneously, of energy, position, and angle with respect to the source. These codes are subject to many uncertainties, including lack of knowledge of basic fast neutron scattering cross sections. This is particularly true in the energy range above 10 Mev. Direct experimental observation of the

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predicted fluxes provides a valuable and all-too-seldom verification. The spectrometer developed and used in this project is particularly oriented toward the high neutron energy region. Data already obtained in water and graphite has been used to guide code developers in the best choice of high energy scattering cross section input.

Publications: No additional publications

RH 00384-03

RADIATION DAMAGE MECHANISMS IN BIOLOGICAL MATERIALS

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Research Objectives: The determination of the nature of the initial damage created by the interaction of ionizing radiation with organic molecules and the subsequent reactions that take place is basic to an understanding of the effects of ionizing radiation in biological systems.

The objectives of the present program are to identify, by electron spin resonance spectroscopy, the initial damage centers formed in a series of biologically important molecules. It is also hoped to detect and identify any intermediate species that are formed during the annealling processes that take place, leading to the formation of the room temperature stable radicals.

Progress Report: Results obtained of the effects of both 220 Kev X-rays and 1 Mev ray on a series of amino acids, organic acids and related compounds indicate that the initial damage centers are created by ionization of an electron from one molecule with subsequent electron capture by a second molecule. However, detailed studies of single crystals of DL Methionine, one of the essential amino acids, have shown that a high concentration of the room temperature stable free radical species is present

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in the crystal immediately after irradiation and measurement at 77°K. Details are given in a forthcoming publication. The conclusions that are reached are that at least in the case of methionine, the annealling processes are rapid, even at 77°K. It was also established that in the case of DL Methionine, the temperature of irradiation had a significant effect upon the nature of the final room temperature stable damage species.

In recent publications (Box, H. C., Freund, H. G., "Confirmations of the Free Radical in the Irradiated α Aminoisobutyric Acid", J. Chem. Phys., 44, 2345-2348 (1966)) Box and Freund describe an electron spin resonance study of α aminoisobutyric acid. In our investigation of this system, we have observed certain differences in the initial damage spectra which appear to be a function of the total dose. A discussion of these findings is being prepared.

One of the major difficulties encountered in the analysis of initial damage spectra is the fact that, in general, more than one free radical species is produced by the ionizing radiation. In amino acids, these various species exhibit similar g factors. For interpretative purposes, this means that the spectra of the different species overlap and hence, definitive analysis is difficult. We have, therefore, been searching for a system in which the initial damage species have sufficiently different g factors that a complete analysis is possible.

A comprehensive study of the free radicals produced by the X-irradiation of single crystals of tetraethylthiuram disulfide was undertaken since the large g factor anisotropy of the sulphur containing radicals enabled a complete analysis to be undertaken. Other systems which have been studied include n propane arsonic acid, histamine diphosphate, O methylisourea, sodium acetate, 3,3'-dimethyl succinic acid and L hydroxyproline. The results of these investigations have either been published or are being prepared for publication.

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There has been increasing evidence that metal ions, particularly copper ions, may mediate the damaging effects of ionizing radiation.

Recently, it has been shown that metallic complexes that protect RNase and trypsin against radiation also proportionately reduce the free radical yields. Present indications are that strong complex formation of the ions is necessary for efficient radiation protection.

The human plasma protein ceruloplasmin has oxidase activity and is known to contain eight atoms of copper per molecule. Both the strong visible absorption, which gives the protein its blue color, and the electron spin resonance signal are associated with the cupric atoms of the molecule. The fraction of the total copper of ceruloplasmin, which in the cupric state is variable, averaging 29%. The activity of the ceruloplasmin has been shown to be dependent upon the presence of these cupric ions.

In preliminary experiments, we have found that both X and γ irradiation causes a complete reduction of the cupric ions to the cuprous state. The cupric ion concentration was monitored by measurement of its electron spin resonance signal. Subsequent oxidation by atmospheric oxygen led to a quantitative regeneration of the cupric ion electron spin resonance signal. However, it was found that the enzyme activity showed only 70% recovery.

We are currently investigating this system in more detail in an attempt to correlate the loss of enzyme activity with changes in the cupric ion protein bonding. This investigation represents a change in the scope of the research. The original objectives were confined to radiation in the solid state. We have recently felt that a significant contribution to our understanding of radiation damage to biological molecules in solution could be made by a combination of electron spin resonance, circular dichroism and magnetic circular dichroism techniques. Using these techniques, it may be possible to relate free radical production and decay processes to more permanent damage to the biological systems, such as metal valence changes in enzymes and conformational changes of proteins and nucleic acids.

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Significance of Research: The use of electron spin resonance and circular dichroism measurements of the damage caused by ionizing radiation permits the development of a basic understanding of the radiation damage processes and associated theory. An understanding of radiation damage mechanisms and possible methods of controlling or minimizing radiation damage is fundamental to the development of means for treatment of radiation sickness or increasing radiation tolerance. From this can come improved application of radiation treatment of disease.

Publications:

Cadena, D. G., Mendez, V. and Rowlands, J. R. "The Electron Spin Resonance Spectrum of CH_3CO_2 Trapped in -Irradiated Sodium Acetate." Molecular Physics, Vol. 13, No. 2, 157-159, 1967.

Cadena, D. G., and Rowlands, J. R. "An Electron Spin Resonance Study of Free Radicals Induced in O-Methylisourenum Chloride by X-Irradiation at 77°K." J. Chem. Soc., B., 1968.

Cadena, D. G. and Rowlands, J. R. "An Electron Spin Resonance Investigation of X-Irradiated DL-Methionine." J. Chem. Soc., B., 1968.

(1) RH 00386-02

RADIATION INDUCED CELL DEATH AT VERY LOW TEMPERATURES

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Research Objectives: This research was directed toward obtaining a better understanding of high energy radiation-induced effects on mammalian reproductive cell death. A comparison was made between the effects of 250 KV X-rays at room temperature and at very low temperatures (-190°C). Such a comparison may permit certain indications of the basic physio-chemical processes involved.

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Progress Report: We have completed a "survival curve" for mammalian cells after exposure to high energy radiation at room temperature and for another set of experiments where the radiation was performed at very low temperature (-190°C). Irradiation at low temperature was less effective (inactivated fewer cells) than did irradiation at room temperature.

The frequency of chromosome aberration produced in these same cells were determined under the same conditions. All experiments were made on primary cultures from Chinese hamster embryos.

A comparison of the two events: reproductive cell death and frequency of chromosome aberration under these experimental conditions strongly suggests that they may be related.

Significance of Research: These data may provide some evidence to a better understanding of how high energy irradiation induces reproductive cell death in mammalian cells. We intend to continue this research.

Publications: None

RH 00392-04

EFFECT OF RADIATION ON PROTEINS AND RELATED COMPOUNDS

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Research Objectives: The aim of this study is to investigate how free radicals interact with biological materials and to investigate the mechanisms of such interactions. Free radicals are generally unstable species because they react quickly with other chemicals and many of them may be expected to affect adversely biological processes. Free radicals are formed on radiation, on heating of certain substances, and in solutions in the presence of various oxidizing agents.

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Progress Report: The effects of ultraviolet radiation on various collagen samples and reference compounds have been studied. Using electron spin resonance spectroscopy, which measures and identifies the free radicals formed, it was shown that free radicals once formed, are able to migrate across molecules until positions of minimum energy were reached. The radicals formed were identified in some instances.

A more quantitative analysis of the ability of the free radicals to migrate within a molecule, or from one molecule to another, showed that such "radical-transfer" reactions occur with surprisingly small energies, at least in some of the model compounds investigated.

The chemical changes, which occur in gamma crystallin, one of the eye-lens constituents, on irradiation in vivo have been compared with the changes observed in gamma crystallin obtained from young and old animals. A new method for determining SH- and -SS- groups was developed, for carrying out such analyses on a sub-micro scale.

Solution studies on model compounds showed that free radicals can sometimes be stabilized through association with other "normal" molecules.

On heating 3,4-benzpyrene, a well known carcinogenic compound present in soot and tobacco smoke condensate, a highly-reactive chemical agent (free radical) has been detected and tentatively identified. Experiments carried out in other laboratories indicate that agents of this type may be more cancer-causing than the unheated 3,4-benzpyrene.

Some of these results have been published in a number of papers in the scientific literature.

Significance of Research: Apart from the exposure of individuals to radiation of various types which gives rise to free radicals, the body is also exposed to a variety of free radicals in other ways, and some of the resulting effects, such as

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cancer formation, may be expected to be similar. The present studies have shown that free radicals are indeed present in substances which human populations are frequently exposed to, such as tobacco smoke condensates. Some information has also been obtained on how irradiation and aging affect certain proteins. The specific purpose of the work concerns itself (i) with identifying potentially harmful free radical species, (ii) with understanding how such free radicals interact with each other and with biological materials such as proteins to give rise to disease, and (iii) with reducing or inhibiting their harmful effects.

Publications:

Forbes, W. F. and Sullivan, P. D. "Organic and Biological Spectrochemical Studies. XXV. Properties of the p-bis (methylthio) benzene and p-bis (ethylthio) benzene cation radicals." Canadian Journal of Chemistry, Vol. 46, 1968.

Forbes, W. F. and Robinson, J. C. "Possible Formation of an Azulene-Type Radical Obtained on Heating 3,4-Benzpyrene." Nature, Vol. 217, No. 5128, 550-551, February 1968.

Forbes, W. F. and Ramsbottom, J. V. "Reactivity of Trapped Free Radicals in Biological Systems." Nature, Vol. 216, No. 5118, 913-914, December 2, 1967.

Forbes, W. F. and Sullivan, P. D. "Free Radicals of Biological Interest, Part II. Electron Spin Resonance (ESR) Spectra of 2537 A Irradiated Amino Acids." Canadian Journal of Biochemistry, Vol. 45 (1967).

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GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION
RH 00394-03	RADIOLOYSIS OF SIMPLE ORGANIC NITROGEN COMPOUNDS	NORMAN N. LICHTIN, PH.D. DEPT. OF CHEMISTRY BOSTON UNIVERSITY BOSTON, MASSACHUSETTS

Research Objectives: Research is directed towards elucidation of mechanisms of radiolysis of simple amides, amines and other organic nitrogen compounds. Interest centers on establishing chemical processes characteristic of each class of compound, on generalizing models of the radiolytic dynamics of polar molecules, and on providing an improved basis for understanding of radiation damage to biologically active molecules. Information on the nature and amounts of stable products, on the influence of dose rate, quality of radiation and scavengers on these, and on the nature and kinetics of observable transient species is being collected.

Progress Report: Initial emphasis has been placed on the radiolysis of liquid formamide, $H-\overset{O}{\overset{\|}{C}}-NH_2$, because of its structural simplicity, its high polarity (dielectric) constant at 25°C is 109), its convenient physical properties and the presence in it of the group $-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{N}}-$, the repeating unit characteristic of proteins. Investigation of formamide is complicated somewhat by difficulty in purifying it and by the fact that it undergoes both hydrolytic and thermal decomposition relatively easily. These technical inconveniences have, however, been overcome and information has been obtained on the nature and yields of stable low molecular weight products of ^{60}Co-gamma radiolysis and the effects on these yields of certain added solutes ("scavengers"). Experiments carried out by us at Brookhaven National Laboratory and the Army Quartermaster Laboratory in Natick, Massachusetts have used very brief but intense bursts of high energy electrons (pulse radiolysis) to provide information on the nature and chemical dynamics of short lived intermediates formed by ionizing radiation.

Only four stable low molecular weight products have been found in significant yields, H_2, CO, CO_2 and NH_3. Their "G-yields", i.e., molecules formed per 100 electron volts

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of energy absorbed are, for neat liquid formamide at room temperature, $G(H_2) = 0.9$, $G(CO) = 0.6$, $G(CO_2) = 0.2$ and $G(NH_3) = 0.3$. The decomposition yield of formamide $G(-HCONH_2)$ corresponding to these product yields is only about 0.9, so low a figure as to suggest that either formamide is relatively insensitive to radiation or, more probably, that substantial yields of higher molecular weight products will be found upon further investigation. Yields of H_2 and CO are insensitive to the presence of dissolved N_2O, a reagent which intercepts hydrated electrons in aqueous solution as well as the solvated electrons produced radiolytically in many other solvents. This indicates that the solvated electron is not a significant precursor of either H_2 or CO. Substantial yields of N_2 are formed, however, $G(N_2)$ varies with concentration of N_2O, reaching a plateau value of 1.9 when formamide is saturated with N_2O at 200 torr or higher. Apparently some reactive species is capable of reducing N_2O but pulse data (cf. below) suggest that this is not the solvated electron. Yields of H_2, CO and CO_2 are also insensitive to added water but yields of ammonia are increased to a degree which is large (about 4-fold) at low doses but decreases with increasing dose. Interpretation of this finding must await investigation of higher molecular weight products.

Information has been obtained in pulse radiolytic experiments by spectrophotometric measurements during and after the pulse. Data were collected at Brookhaven using a pulsed beam of 1.15×10^6 V electrons produced by a Van de Graaf accelerator. Most of the data were based on 20 microsecond pulses although some important kinetic information was obtained with 4 and 8 microsecond pulses. The transient spectrum between 300 and 600 nm under these conditions is characterized by an intense maximum at 400 nm, a shoulder centered near 550 nm and absorption which diminishes at higher wavelengths. The 550 nm absorption decays much more rapidly than that at 400 nm. This decay follows first order kinetics characterized by a half-life of 2×10^{-6} sec. Decay of the 400 nm species is second order in the absorbing species. Since the molar absorbancy index, e, of the transient species is not known, only the value of k/e, where k is the specific rate of the reaction, can be calculated from the data. This was found to be 1×10^6 cm sec $^{-1}$. The data

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establish the formation of at least two transient species, neither of which absorbs at wavelengths characteristic of solutions of solvated electrons in water, ammonia or alcohols. Measurements carried out at the Army Quartermaster Laboratory in Natick, Massachusetts, employed a pulsed beam of 7×10^6V electrons produced by a linear accelerator. The dose rate (energy per unit time) of these pulses was more than ten times that provided by the Brookhaven Van de Graaf but the pulse period was only 1.6 $\times 10^{-6}$ sec. so that energies per pulse were similar in the two sets of experiments. The transient spectrum was studied in this case between 300 and 700 nm. Significant absorption is not observed above 600 nm. The 400 nm peak was reproduced but, under these conditions, the 550 shoulder appears as a distinct peak almost twice as intense as that at 400 nm, a result which is consistent with the kinetic analysis of the Van de Graaf data. Neither the spectrum nor its kinetics of decay is affected greatly by saturation of formamide with 1 atm. of N_2O, a result which indicates that neither transient is the solvated electron. Saturation of formamide with 1 atm. of N_2O, a result which indicates that neither transient is the solvated electron. Saturation of formamide with 1 atm. of O_2 replaces both peaks with much less intense absorption. Pulse radiolysis of aqueous solutions of deaerated formamide under an atmosphere of argon gives a transient spectrum devoid of the intense maxima observed in neat formamide. Assignment of the observed transient absorptions to various chemical species has not yet been accomplished. The difference in the nature of the transients in neat formamide and in aqueous solution is particularly significant.

Significance of Research: The achievement of understanding and control of radiation damage to living organisms is a fantastically complex problem involving the interaction of many subdisciplines of physics, chemistry and biology. Among the many kinds of fundamental scientific information essential to this task is detailed and sophisticated knowledge of the chemical changes brought about in biologically active molecules by ionizing radiation. Such molecules, particularly proteins and nucleic acids, are, however, themselves so complex that it is necessary to investigate the radiation chemistry of much simpler model compounds in order to identify and fully

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delineate the relevant radiolytic processes. The research of this program is devoted to a searching study of the radiation chemistry of the simplest molecules which can be regarded as related to biologically active compounds.

Publications: None

RH 00433-02

RADIATION STUDIES IN IONIC MATRICES

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Research Objectives: Simple inorganic and organic ions, which are present in biological systems or which can serve as chemical models of more complex biological compounds, are dispersed in solid potassium halide matrices, and these solids are exposed to ionizing radiations. The chemical changes of the solute ions produced in these samples will be characterized by measuring the infrared, ultraviolet, visible, and/or electron paramagnetic resonance spectra of the samples.

Progress Report: In ordinary bicarbonate salts, the bicarbonate ions are present as hydrogen-bonded cyclic dimers, $(HCO_3^-)_2$, or linear polymers, $(HCO_3^-)_n$. The chemical properties of these polymers as well as their structures are quite complex, and it does not seem likely that irradiation of these salts will lead to an understanding of the processes involved in the radiation damage of even a simple substance like the bicarbonate ion. However, when these bicarbonate salts are dispersed in solid potassium halide matrices and then these diluted systems are heated, the salts are converted almost completely into monomeric bicarbonate ions trapped in the matrix. The chemical reaction of this monomer ion is relatively simple and so is its radiation chemistry.

When HCO_3^- trapped in a KCl matrix is heated, an unexpected product formate, HCO_2^- is formed. The rate of disappearance of the bicarbonate ion is found experimentally to

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be the same as the rate of appearance of the formate, and both rates depend only on the concentration of the bicarbonate monomer ion present at any instance. Thus, we can conclude that the formate is a primary decomposition product of bicarbonate and not of any other substance which may be present in the solid. Gamma ray induced decomposition of bicarbonate, on the other hand, produces the carbonate anion free radical, CO_3^- , as the major product and the carbon dioxide anion free radical, CO_2^- , as a minor product. Both of these ions are paramagnetic, and they can easily be detected by electron spin paramagnetic resonance spectroscopy. The principal radiation damage reaction, therefore, is $HCO_3^- = H + CO_3^-$, and the hydrogen presumably diffuses out of the solid matrix as gaseous molecular hydrogen. Typically, with 0.05 milligram of potassium bicarbonate in 0.5 gram of potassium chloride solid about 1.5×10^{16} carbonate anion radicals were formed. At room temperature, these radicals disappeared with a half-life of 44 hours (2.8 hours in KBr matrix), and the decay products were diamagnetic. Since the decay rate depended on the concentration of the radical raised to the second power, the decay product must be either diperoxycarbonate ion, $C_2O_6^=$, or $CO_2 + O_2^=$. Matrices containing the decay products showed a positive test for peroxide. Present experimental evidence, although not conclusive yet, favors the diperoxycarbonate as the decay product of the carbonate anion free radical.

Although a small amount of CO_2^- radical was produced during the gamma irradiation of bicarbonate, the best method to generate this radical ion is by the irradiation of formate ion trapped in potassium halide matrices. In this case, the principal degradation reaction is $HCO_2^- = H + CO_2^-$ and the hydrogen presumably diffuses out of the matrix as molecular gas as in the case of bicarbonate. The concentrations of CO_2^- which can be produced by our method are high enough that chemical studies can be made. We find from these studies that the radical ion reacts very rapidly with water to produce formate and bicarbonate ions. However, in the absence of traces of moisture the radical decays very slowly and two CO_2^- ions are involved in the decay reaction. (The decay of CO_3^- radical is about 10^4 times faster than the decay

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of CO_2^-.) The decay products, therefore, must be either oxalate, $C_2O_4^=$, or CO + $CO_3^=$. This conclusion is consistent with an earlier observation that small amounts of formate and bicarbonate ions were invariably formed during the thermal decomposition of the oxalate ion.

Bicarbonate, formate, and oxalate are not the only simple ions whose chemistry is related through the radical CO_2^-. This radical was detected when the acetate ion was irradiated. Methane and ethane were also detected in this case. Thermal decomposition of the acetate, on the other hand, gave formate as the major product. Although the stoichiometry of the latter reaction has not yet been completely established, two acetate ions were found to produce one formate ion. Thus, it appears that in both the thermal decomposition reaction and the radiation damage reaction of the acetate ion, the process $CH_3CO_2^- = CH_3 = CO_2^-$ is involved. If this is the case, then there is a possibility that other reactive species such as CH_2 or $CH_2CO_2^-$ may be trapped in our matrices. However, radicals other than CO_3^- and CO_2^- have not yet been identified conclusively in irradiated acetate systems.

Other ions we are investigating are the cyanate ion (OCN^-) which is one of the most common impurities we have observed when commerical inorganic and organic salts are heated, the fumarate ion ($^-O_2C\text{-}CH{=}CH\text{-}CO_2^-$) which will be used as a model for the study of the radiation chemistry of a double-bonded system, and the perchlorate ion (ClO_4^-) which will provide an oxidizing condition during some of our solid state irradiation studies. Quantitative and interpretable results are being obtained with all of these ions.

Significance of Research: When matter is exposed to ionizing radiations, chemical degradation occurs, and as the name of the radiation implies, many of these degradation products are ions. In our studies, we seek not only to identify such ions but to study their chemical properties as well. Since the scientific literature suggests that radiation studies with actual biological compounds have not produced or are not producing the desired chemical information, our studies employ instead

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simple but biologically significant inorganic and organic ions. Ions such as bicarbonate, formate, or acetate have been selected for investigations because these ions are actually present in some of the biological systems and because they can also serve as simple chemical models of the more complex biological compounds. In order to differentiate between ions formed initially by the radiation and those formed by subsequent reactions, our radiation samples are very dilute solid solutions of parent ions in relatively inert solvent of potassium halides. In spite of the simplicity of our experimental technique and approach, our studies are producing chemical data which have not been available previously.

Publications: None

RH 00450-03

TRANSFER MECHANISMS IN IRRADIATED BIOLOGICAL SYSTEMS

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Research Objectives: Our objective is to improve our understanding of the chemical changes which are caused by ionizing radiation. Molecular damage produced by ionizing radiation is often transferred from one part of a molecule to another part or even from one molecule to another molecule. In this investigation such transfers of damage are being studied in a variety of biological molecules. A form of spectroscopy known as magnetic resonance spectroscopy is used to observe the transfer processes.

Progress Report: The primary step in the chemistry of radiation damage is the ionization of molecules by the radiation. At normal temperatures these ionized molecules have a very transient existance and very quickly react to produce neutral damaged species. Thus the ionized stage of the radiation damage process is normally not observed. A method for studying the ionic phase of the radiation damage process was developed at this laboratory. It consists of irradiating the sample at very low

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temperature, -450°F or lower. At this low temperature the ionized stage of the radiation damage process is "frozen in".

Ions stabilized in this manner have been studied by electron spin resonance spectroscopy. This kind of spectroscopy is ideal for our purpose because it measures an absorption due to the ionized molecules, usually present only about one part in a million, and is oblivious to all the normal molecules which have not been affected by the radiation. The unique absorption of the ionized molecules is due to the fact that a molecule becomes paramagnetic upon loss of an electron (that is to say upon being ionized). Likewise, the molecule which picks up the dislocated electron also becomes paramagnetic.

From paramagnetic studies of ionized molecules stabilized at low temperature, we have demonstrated that a rapid transfer of holes takes place within molecules. That is to say, the electron stripped off a molecule by the ionization process may come from any atom in the molecule. But the electron vacancy or "hole" is quickly shifted intramolecularly to a specific site in the molecule. This preferred site for the hole usually becomes the focus for further reactions.

By allowing the sample to warm the next phase of the radiation damage process can be observed. Often the ionized molecule degrades into broken molecules called radicals. At still higher temperatures another transfer process, this time between molecules, may occur. Intermolecular transfer processes usually involve the transfer of atomic hydrogen and can be followed by magnetic resonance spectroscopy. Using these methods, we are able to observe how radiation damage becomes localized at specific molecular sites and how damage can be transferred between molecules in a mixture of molecules.

Significance of Research: The most significant application of this research is to the problem of radiation protection. For example, it has been possible to show how

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RH 00450 (Continued)	a small amount of cysteine, a well known chemical radiation protector, is able to mediate radiation effects. Other radiation meadiators are being tested in the same way. Publications: None	
RH 00464-02	ESR ABSORPTION OF IRRADIATED ORGANIC CRYSTALS	ICHIRO MIYAGAWA, DR.S. DEPT. OF PHYSICS UNIVERSITY OF ALABAMA UNIVERSITY, ALABAMA

Research Objectives: With the use of the electron spin resonance (ESR) technique, this investigator and his associates have discovered that chemical reactions in X-ray irradiated crystals of some organic compounds are very similar to enzymatic reactions. This project is intended to clarify the nature of these reactions. Since no such reactions are observed in ordinary organic reactions, it is hoped that this investigation will provide unique knowledge which will be useful for interpretation of the mechanism of radiation damage of biological systems.

Progress Report: (1) Unusual Exchange Reaction. We have discovered that the C-H hydrogens of the radicals in irradiated crystals of amino acids exchange easily with deuterium of the host crystals. This exchange reaction is unusual for the following reasons. Exchange of the C-H hydrogens of amino acids takes place only with difficulty: according to biochemist Oshima, when an amino acid in a strongly acidic or alkaline solution was heated in an autoclave at 110°C for a week, only 20% of the hydrogens were exchanged. Physical chemist Garnett studied exchange reactions of amino acids catalyzed by platinic oxide: even at high temperatures the exchange was never complete. It should be noted that chemist Fischer observed no evidence of exchange of the C-H hydrogens in the case of a chemically produced radical CH_2OH. However, biochemists Tamiya and Oshima had discovered an inter-

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esting phenomenon: the C-H hydrogens of an amino acid are exchanged easily with the aid of a small amount of an enzyme. This enzyme is transaminase which catalyses transfer of an amino group from one amino acid to another. Thus, similarity between the exchange reaction in irradiated crystals and that catalysed by the enzyme is unusual.

In the present project, a new exchange reaction was discovered: the exchange takes place between the C-H hydrogens of a radical and the C-H hydrogens of the adjacent molecules in an irradiated crystal of L-alanine. The experimental evidence is as follows: Radicals from a deuterated L-alanine, $CH_3CD\ ND_3^+\ CO_2^-$, are a mixture of 22% $CH_3CD\ CO_2^-$ and 78% $CH_3CH\ CO_2^-$ rather than 100% $CH_3CD\ CO_2^-$. Hence, the deuterium of the initially produced radical $CH_3CD\ CO_2^-$ must exchange with the hydrogens of the CH_3 groups of the adjacent molecules which are the only source of the protons. The rate of exchange was so fast that only the exchanged mixture of the radicals were detected. This rate is much faster than the corresponding enzymatic reaction which takes about 1/2 hour to complete at 35°C. The previously discovered one is much slower compared to the enzymatic reaction.

(2) High Stereospecificity. We have discovered that hydrogen (deuterium) addition to fumaric acid in an irradiated crystal of succinic acid is highly stereospecific, and the ratio of concentration of DOO $CD_\beta H_\beta$' CHCOOD to that for DOO C $CH_\beta D_\beta$'CHCOOD is higher than 300 : 1. We have also discovered that in an irradiated crystal of aspartic acid the rate of exchange of the two beta hydrogens of the radical HOOC CH_2CH COOH is 20 : 1. So far as we know, a high stereospecific efficiency, such as the above-mentioned one, has been found only in the case of enzymatic reactions. Most of the pure organic reactions show no evidence of such stereospecificity: for example, a D-compound and its L-compound are produced with equal probability.

It has been shown that when a crystal of L-alanine is irradiated at liquid air temperatures, a negative ion is produced and subsequently a proton combines with

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the ion. In the present study it was found that this proton addition is highly stereospecific: the addition takes place specifically for one of the two oxygen atoms of the CO_2^- group of the ion of L-alanine. The mechanism of this addition reaction is much simpler and clearer than the case of fumaric acid, and hence we are trying to analyse the stereospecific efficiency theoretically. For this reason, we believe that this result is important for clarification of the nature of the stereospecificity.

(3) Development of a New Sample Cavity. We developed a microwave cavity which is convenient for the study of single crystals irradiated at liquid air temperatures. The cavity which is made of copper is a TE_{111} cylindrical one for 9.2 kMc/sec. A crystal fixed on the metal piece which serves as a crystal holder is transferred to the cavity, after being irradiated. Any orientation of the irradiated crystal is obtained by rotation of the crystal holder and rotation of the magnet. Although the system is immersed in the liquid air, the cavity is filled with gaseous air which is supplied by evaporation of the liquid air. Thus, the cavity is free of bubble noises. A 40 kV X-ray source can be used to irradiate crystals at liquid air temperatures. Expensive and dangerous sources, such as high energy X-ray sources or cobalt sources which have been used in most of the works at low temperatures, are not needed. Moreover, the device allows an accurate measurement of temperature of the irradiated crystals over the range from 80°K to room temperature.

Significance of Research: Our knowledge on similarities between enzymatic reactions and chemical reactions in irradiated organic crystals is highly limited. Our research suggests, however, a possibility that some of the chemical reactions which are catalyzed only by enzymes can be activated by ionization irradiation, when a biological system is irradiated. It should be noted that chemical reactions in a biological system are a coordinated combination of a number of enzymatic reactions which are more or less centrally controlled. Thus, if ionization irradiation activates a chemical reaction without the aid of the enzyme, the biochemical balance of the

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RH 00464 (Continued)	biological system would be disturbed. Such a disturbance, if it occurs, undoubtedly has a harmful effect on the biological system. Publications: None	

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RH 00204-06	BIOLOGICAL EFFECTS OF LOW VELOCITY ELECTRONS	GEORGE S. SPERTI, SC.D. DIV. OF PHYSICS & BIOPHYSICS INSTITUTUM DIVI THOMAE CINCINNATI, OHIO

Research Objectives: The researches being conducted under this grant are planned:

a. to give an insight into the method by which electrons produce some of their biological and biochemical effects; and

b. to determine if and how the mechanism of action of electrons and electromagnetic radiations (such as those emitted by radioactive materials) on biological and biochemical substances are related.

Progress Report: A low velocity electron generator for use in biological and biochemical research has been developed. The unit is constructed in a manner which allows for rapid determination of the intensity and homogeneity of the electron beam. Without disturbing the vacuum, three different specimens may be bombarded in succession, thereby assuring uniform conditions where comparative data are desired.

Results of experiments on the bombardment of bacteria and yeast with electrons accelerated by potentials ranging from 0 to 750 volts show that these organisms are killed at threshold electron velocities. Staphylococcus albus, which has an average size of 0.7-0.9μ, required electron accelerating potentials of approximately 25 volts and over to produce a lethal effect, while Saccharomyces cerevisiae, with an average diameter of 50μ, is killed only by electrons which have been accelerated by potentials in excess of 300 volts and for the entire energy range tested. The experiments also establish the fact that the energy required to kill yeast cells is approximately 12 times that required to kill S. albus.

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Earlier experiments by one of the investigators, using ultraviolet radiations, established the fact that S. albus and Saccharomyces cerevisiae are killed by the same critical wave length, vis., 2960 Å. This, however, corresponds to the energy of an electron accelerated by a potential of only 4.12 volts. In electron bombardment of organisms the loss of energy of the electron in penetrating the cell to the depth where it becomes effective might account for the fact that the quantum required in the form of electrons is larger than that in the form of radiations. Since the absorption of electrons and radiations by cellular components is different, the two agents may be acting on different portions of the cell. However, the results suggest the possibility that there is an inherent difference in the mechanism of action of radiations and electrons.

Since sufficient data are not available for the accurate determination of the absorption of low velocity electrons in solids, interpretations of their mode of action on microorganisms are hardly justifiable. However, the results do indicate that the lethal action of electrons below 500 volts is in all probability a surface effect.

By bombarding a thin, uniform film of an enzyme, the penetrating power of the electrons has been estimated by determining the maximum percentage of enzyme that can be inactivated with electrons of a given velocity.

Thin films of trypsin were exposed to electrons accelerated by 500, 400, and 300 volts at energy densities of 1.3, 2.6, 3.9, and 5.2 joules/cm^2, and at 200 and 100 volts to the maximum energy attainable in the gun. The percent loss of activity at 500 volts reaches a maximum when the enzyme is subjected to an energy of 2.6 joules/cm^2, and remains constant at all the higher energy levels. This corresponds to the maximum penetration power of the electrons. Using the weight of the enzyme thus destroyed by the electrons, the area of exposure, and assuming the density of a protein to be 1.3 g/cm^3, the depth to which the electrons penetrated the enzyme film was calculated.

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The penetrating power was determined for electrons accelerated by potentials ranging from 100 to 500 volts. Two hundred volt electrons had an average range in trypsin of approximately 60 Å while 500-volt electrons traveled an average of 140 Å. It is interesting to note that 100-volt electrons produced no inactivation. It is highly probable that at this voltage the lack of inactivation was not due to the failure of the electrons to penetrate the enzyme film but rather to the fact that at 100 volts an electron does not possess enough energy to adversely influence a trypsin molecule.

Significance of Research: Although the beneficial and harmful actions of electrons and radiations emitted by radioactive substances have been the subject of investigations for many years, the method by which they produce most of their effects is not understood.

In using high velocity electrons to bombard cells, it is as though one were shooting an animal with a high velocity rifle bullet capable of injuring many organs. The end result could be death, but how the shot brought about death may not be understood. On the other hand, if the animal were shot with a rifle bullet of the lowest velocity that would kill (corresponding to a low velocity electron) it could be determined if death resulted from injury to the heart, brain or other organ. Clearly, the lowest velocity bullet which would kill could yield the most revealing information.

It is hoped that through the research conducted under this grant, in the area of low velocity electrons, the beneficial action of radioactive substances may be better understood and utilized, and the harmful actions more effectively controlled.

Publications: No additional publications.

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RH 00302	LASER RADIATION IN PHOTOCHEMISTRY	KARL WEISS, PH.D. DEPT. OF CHEMISTRY NORTHEASTERN UNIVERSITY BOSTON, MASSACHUSETTS

Research Objectives: This research is concerned with the elucidation of the nature of specific photochemical effects in biological damage caused by laser radiation. The technique of laser flash photolysis is used to study model chemical systems such as dyes and charge transfer complexes, as well as biologically active molecules. Laser-induced photochemical changes brought about by multiphoton absorption under conditions which preclude direct (one-photon) excitation are also under investigation.

Progress Report: We have developed the apparatus for laser flash photolysis, which provides photochemistry and photobiology with a unique new investigative technique. The absence of stray light and the shortness of the laser pulse make it possible to detect and characterize short-lived intermediates which cannot be observed by conventional flash methods.

This technique has been successfully applied to the study of methylene blue, a dye which is widely used to sensitize the photo-oxidation of amino acids, proteins, purines, and nucleic acids. This dye is known to exist in equilibrium with its dimer, and the proportion of monomer and dimer depends on the concentration of the solution. The giant pulse ruby laser photolysis of aqueous solutions of methylene blue revealed three transient species with lifetimes in the range 2-140 microseconds, which have been characterized and identified. There was evidence that saturation of light absorption occurs and that the initial conversion into an excited state is complete even in very concentrated dye solutions. This means that the laser can penetrate optically dense media which transmit no light at low intensity.

The photochemical results are significant in that they indicate that both the monomeric and dimeric forms of methylene blue give rise to transient intermediates by separate

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excitation. The participation of polymeric dye forms in photochemical processes appears to have been largely overlooked by other investigators. It may have important implications in the photochemistry of dyes. The longest-lived transient (half-life 140 microseconds) is insensitive to oxygen, and has been assigned as a dimeric charge transfer state which is considered to result from the transfer of an electron from one dye molecule to another within the excited state of the dimer. This concept of the relaxation of polymeric excited states may well be applicable to systems other than dyes. For instance, the well-known excited dimers of polynuclear hydrocarbons may decay via this type of intermediate.

Ruby laser-excited transient intermediates have been observed with charge transfer complexes, with aqueous copper ions, and with the sodium ketyl of benzophenone. Experiments with the charge transfer complexes are still in progress. The early experiments with methylene blue and with the metal ketyls revealed decay profiles (optical transmission versus time at constant wavelength) on which regular sinusoidal patterns are superimposed. With the dye solutions, the amplitude of the pattern depends on the pathlength of the sample cell. We have not been able to develop a satisfactory explanation for this pattern. Since it interferes with the data evaluation, we have taken the pragmatic approach of finding conditions under which the pattern is supressed. We recognize, nonetheless, that this effect may be worthy of more detailed study since it might reflect an energy conversion mechanism which is capable of producing biological damage.

Under the extremely high photon densities attainable with the pulsed laser, multiphoton absorption can occur. Since double photon absorption of laser radiation is equivalent to the absorption of a single photon with twice the energy of the laser photon, this process offers the possibility of bringing about photochemistry in the interior of structures which are transparent at the laser wavelength at low intensity. We have conducted laser experiments with several compounds in solution under multiphoton conditions, and have demonstrated that short-lived intermediates can be generated and studied by kinetic spectrophotometry. With the hydrocarbon anthracene, transient

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absorption was observed in the region where its triplet excited state absorbs, and it is estimated that ca. 0.5% of the incident laser light is absorbed by a two-photon process under the conditions employed. The hydrocarbon naphthalene, in very concentrated solution, also gives rise to a transient species under multiphoton conditions which, however, does not correspond to the known triplet excited state of this compound. With the ketone benzophenone, the integrated effect of a large number of laser pulses produced a chemical change, although no transient absorption could be detected. The systematic investigation of these model systems is continuing. We have also planned a comprehensive series of multiphoton experiments with biologically significant compounds such as amino acids, enzymes, flavins, DNA, nucleic acid bases, and the visual pigments.

With an excitation laser pulse of ca. 20 nanoseconds duration, transient intermediates with lifetimes < 100 nanoseconds ought to be amenable to measurement. This is presently not the case owing to limitations of our current detection circuit, which provides a time resolution in the microsecond range. We have designed, and are in the process of constructing, a new detection circuit which is based on a photodiode and a multistage pulse amplifier, and which is anticipated to provide a time resolution ~5 nanoseconds. The new device should enable us to study very short-lived triplet states such as those involved in cis-trans isomerization, and possibly even some excited singlet states.

Significance of Research: Although much work has been done on the biological effects of laser radiation, there is no detailed knowledge about the interactions at the molecular level. Damage may result from conversion of the radiation into highly localized thermal energy, from mechanical effects and pressure waves, from electric field effects, and from reactions involving photochemically excited states. Information is needed about the molecular components which render structures susceptible to laser radiation, about intra- and intermolecular energy transfer, about the nature of the chemical reactions which occur, and about sensitization mechanisms. This study is concerned with these basic aspects of laser biological damage, with particular emphasis on photochemical effects.

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The phase of this project which deals with multiphoton processes is of great potential significance with respect to the health hazards associated with non-ionizing electromagnetic radiation. The magnitude of the changes produced by multiphoton absorption increases in a non-linear fashion with the intensity of the irradiation. Consequently, biological multiphoton effects are liable to be most pronounced at the intensity levels attained by pulsed lasers in industrial, clinical, and research applications, and by the explosion of nuclear devices.

Publications:

Schnieper, P. A. "Flash Photolysis - An Application of Electrical Engineering in Chemical Research." NEREM Record, 158 (1965), a Digest of Papers presented at the Northeast Electronics Research and Engineering Meeting on November 3, 4 & 5, 1965.

Danziger, R. M., Bar-Eli, K. H. and Weiss, K. "The Laser Photolysis of Methylene Blue." Journal of Physical Chemistry, Vol. 71, 2633, 1967.

(3) RH 00506-01

BIOCHEMICAL CHANGES IN SKIN INDUCED BY IRRADIATION

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Research Objectives: A systematic investigation into the biochemical alterations produced by sunlight and ultraviolet light irradiation on human, intact skin presents a challenging research problem in the basic sciences and in dermatology. There are numerous studies describing morphologic, gross and microscopic, changes as well as histochemical alterations subsequent to the skin's exposure to ultraviolet light. However, biochemical analyses of the major epidermal components remain limited.

The following research problem is designed to permit a more comprehensive, better controlled investigation of alterations in skin constituents as related to irradiation

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exposure. In particular, careful attention will be given to biochemical modifications which may give some clue to the mechanisms in re

1. the capacity of irradiation and environmental factors to injure skin with the ensuing production of erythema, inflammatory response, promotion of pigment formation, acceleration of degenerative changes and of premature aging,

2. the relationship of ultraviolet light to photosensitivity and its role in precipitating or aggravating systemic diseases such as lupus erythematosus, pellagra, porphyria and photosensitization phenomenon associated with certain drugs,

3. the influence of sunlight and ultraviolet light on enzyme activity and the formation of substances with potent biochemical and physiological actions both beneficial (e.g., formation of Vitamin D) and deleterious (e.g., formation of premalignant and malignant skin morphology).

Hopefully, the research project outlined above will contribute to a better understanding of this complex subject of photobiology.

Publications: None

(3) RH 00530-01 BEHAVIORAL EFFECTS OF LOW INTENSITY UHF RADIATION

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UNIVERSITY OF ARKANSAS
FAYETTEVILLE, ARKANSAS

Research Objectives: While the somatic effects of ultra-high-frequency (UHF) radio waves at high power and high frequency levels have been investigated extensively, the effects at low power and low frequency levels have largely been neglected. Investigations of the latter sort are needed since modern communication systems use low power and low frequency UHF levels.

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Therefore, the major objectives of the proposed project include investigations of the following variables under conditions of low level UHF radiation: (1) Behavioral effects: Although the author has already established that the behavior of rats is modified by low-level UHF, e.g., changes in activity, emotionality, and susceptibility to seizures, the presently proposed project will involve the investigation of other possible UHF-induced behavioral changes (adaptability of behavior, learning, stress, as well as further measures of activity and emotionality). (2) Power density levels and duration levels: The second major objectives is the exploration of possible differential behavioral effects (a) under three levels of low power density (9, 5, and 1 mw/cm^2) and (b) after three durations of exposure (3, 6, 9 mos.). (3) Neurophysiological substrates underlying behavioral effects: Although it has been assumed that the mechanism underlying UHF effects is heating of body tissue, recent evidence points to a particular neurohumoral system. The proposed drug studies and biochemical assays would provide information concerning the role of this system.

In summary, the broad objective of the proposed research is to obtain information concerning the effects of low-level UHF at the behavioral level and at the physiological level, as well as identifying the physical stimulus correlates associated with these levels.

Publications: None

BENEFICIAL APPLICATIONS OF RADIATION

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RH 00061-13	IMPROVEMENT IN SANITATION BY IONIZING RADIATION	SAMUEL A. GOLDBLITH, PH.D. DEPT. OF NUTRITION & FOOD SCIENCE MASSACHUSETTS INSTITUTE OF TECH. CAMBRIDGE, MASSACHUSETTS

Research Objectives: The prime research objective is to develop means and methods of improving sanitation by employing ionizing radiation. The research has centered around increasing the storage life of perishable food products. Also, methods and processes are being developed that will make public-health significant organisms more sensitive to ionizing radiation.

Progress Report: (1) Food Applications. With regard to practical applications of ionizing radiation, a study has been completed on the combined effects of ionizing radiation and storage in a carbon dioxide atmosphere on the extension of the storage life of haddock. Irradiated and unirradiated haddock fillets were stored in air and in 100 per cent carbon dioxide at 37°F. The unirradiated fish in an air environment had a storage life of four days, while the samples stored in 100 per cent carbon dioxide had a storage life of 15 days. Samples irradiated with a low dose and stored in air had a storage life of 17 days while those stored in carbon dioxide had a storage life of 22 days.

(2) Radiation Resistance of Microorganisms. The radiation resistance of microorganisms varies greatly. Within a given genus, i.e., Micrococcus radiodurans versus Micrococcus roseus, the radiation resistance can vary greatly. A study was conducted to explain on a chemical basis the differences in the observed radioresistance between organisms. Four highly radioresistant strains of M. radiodurans and two radiosensitive strains of M. roseus were examined. Due to the lack of any significant differences in chemical composition including differences in the DNA content or base ratios of the strains investigated, it was postulated that the high radioresistance of the M. radiodurans strains is probably due to an efficient repair mechanism.

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(3) Sensitization of Microorganisms. Investigations on methods and chemicals that may sensitize microorganisms to radiation are being conducted. Work with napthoquinones (a class of sensitizer such as Vitamin K_5) has shown that if these compounds are present during irradiation, the dose required to inactivate the organisms is reduced. It is thought that these compounds react with the cell and prevent the cell from neutralizing or overcoming the effects of ionizing radiation.

(4) Hospital Supplies. Another practical application of ionizing radiation that is being completed is a study on the inactivation of common contaminants of medical supplies by ionizing radiation. It has been found that if the moisture in the air (per cent relative humidity) is at a certain level, microorganisms likely to be contaminating medical supplies can be more effectively inactivated. This is an important consideration in the proper sterilization of medical supplies.

Significance of Research: There are many factors which contribute to the radiation resistivity of microorganisms. Some factors seem to be environmental, while others appear to be the result of an inherent resistivity. The inherent resistivity in some cases may be due to an effective repair mechanism. The significance of this research is that it may separate these factors and allow for a more effective application of ionizing radiation to biological materials for preservation and sterilization.

Publications: No additional publications.

RH 00311-05 — IRRADIATION AND MARROW TRANSPLANTATION IN LARGE ANIMALS

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DEPT. OF MEDICINE
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

Research Objectives: The primary objective of this research project is that of protecting against the lethal effects of whole body irradiation by bone marrow transplantation. Two major and necessary correlative studies include:

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1. Methods of maintaining life during periods of bone marrow failure. These techniques include maintenance of electrolyte and water balance, prevention and control of infection, provision of platelets and provision of large numbers of granulocytes.

2. Studies of the immunological complications of marrow transplantation. These include studies of the nature of the immunologic reactions responsible for graft rejection or graft-versus-host disease and studies of means of control of immunologic reactions using immunosuppressive drugs, antilymphocyte serum and extracorporeal irradiation.

Progress Report: 1. Isogeneic marrow grafts. Isogeneic grafts in which the tissue donor and recipient are of the same genetic composition. These include autografts, grafts between identical twins and grafts between inbred animals. Isogeneic grafts are generally successful since there are no immunological problems. We have found that dogs can regularly be protected against supra-lethal doses of whole body irradiation (600 to 1800 r) by infusions of their own marrow set aside before the irradiation. These autografts permit studies of techniques of long-term preservation of bone marrow and studies of long-term effects of whole body irradiation.

Isogeneic marrow grafts in human beings are effective if an identical twin is available to serve as marrow donor. In three instances in Seattle, patients with acute bone marrow failure have recovered promptly after an infusion of marrow from an identical twin. In October of 1967, an irradiation accident occurred in Pittsburgh in which one technician received a whole body dose of 600 r. Fortunately, the man had an identical twin. The principal investigator went to Pittsburgh and performed a marrow transplant which was followed by rapid hematologic recovery of the accident victim.

2. Allogeneic marrow grafts. Allogeneic grafts are grafts between individuals of different genetic composition. Here immunologic reactions, host-versus-graft or

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graft-versus-host, occur regularly. These immunologic problems constitute the major barrier to the widespread application of tissue grafting, not only of bone marrow, but of other organs such as kidney and heart. Our studies in the dog have shown that a long-term marrow graft can be achieved following 1500 r of whole body irradiation about one time in ten when donor and recipient are selected randomly. If the immunosuppressive drug, methotrexate, is given in the first ten days after marrow grafting the success rate climbs to 30 to 40%. Two male dogs were irradiated with 1500 r of whole body irradiation and then given a single marrow transplant from female donors. Four years later cytogenetic analysis of the chromosomes of cells from the peripheral blood, the bone marrow and the lymph nodes all showed 100% female karyotypes. These animals continue in good health. Long-term observation of these animals and others similarly treated is continuing.

3. Histocompatibility typing. As indicated above, when the marrow graft donor and the recipient are selected at random, the result is quite variable, ranging from successful long-term chimerism to early death from severe secondary disease. This variability is presumed to be the result of differing degrees of antigenic incompatibility between donor and recipient. Histocompatibility typing represents an attempt to select compatible donors and recipients prior to grafting. In the dog, we have shown that matching for red cell groups does not influence the outcome. For the past several years we have worked on the problem of lymphocyte typing using the cytotoxicity test. Four reasonably specific typing sera have been developed. Within litters these typing sera have allowed us to select matched pairs that regularly become long-term radiation chimeras in contrast to the early death of mismatched pairs. In unrelated dogs, the sera are of definite but limited value in predicting success or failure of a marrow graft but additional sera are needed for complete typing of unrelated animals. Recently, we have adapted the mixed lymphocyte reaction to histocompatibility typing in the dog and results indicate good agreement between the cytotoxicity typing and the mixed lymphocyte reaction. The value of the latter test in marrow grafting is currently being assessed.

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(Continued)

4. Extracorporeal irradiation. Extracorporeal irradiation is a technique that permits suppression of immunologic response by destroying lymphocytes outside the body without the hazards of whole body irradiation. Our studies in primates, the baboon, have shown that extracorporeal irradiation can achieve a striking reduction in lymphocytes associated with markedly prolonged survival of skin grafts.

The technique for extracorporeal irradiation developed in our laboratory involves the use of the Scribner type silastic-teflon shunt which permits extracorporeal irradiation without anticoagulants and without the use of a pump intermittently or continuously. Solution of the technical problem has made it possible to carry out studies of the effect of extracorporeal irradiation in various types of human leukemia, particularly chronic lymphocytic leukemia. Six patients, refractory to conventional therapy, have been treated with a striking reduction in lymphocyte counts in all patients. Four of the patients have returned to pretreatment levels in three to six months. Two did remarkably well, however, and one such patient has now been managed by extracorporeal irradiation alone for a period of almost four years.

5. Studies of cross circulation. We have also adapted the teflon-silastic shunt technique to cross circulation and have shown that cross circulation can be carried out for long periods of time without anticoagulants and without the use of a pump. Immunosuppressive drugs have been evaluated in the dog and the baboon. We have shown that cross circulation is an effective way of maintaining the platelet count and of providing large quantities of granulocytes to the animal with radiation induced bone marrow failure. Cross circulation has also been shown to prolong skin graft survival. Marrow grafts have been achieved by cross circulation through the effect of the stem cells present in circulating blood.

Cross circulation has now been used in eight human cases either for bone marrow failure or for hepatic coma. In one patient with acute liver failure, life was maintained for ten days by cross circulation with eventual complete recovery of the patient. One patient with leukemia, treated with 300 r of whole body irradiation,

BENEFICIAL APPLICATIONS OF RADIATION

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00311 (Continued)

was supported with platelets and granulocytes provided by cross circulation for a period of four months. Additional studies of the usefulness of cross circulation in human beings with irradiation induced marrow failure or with other types of c· an failure are planned.

6. Immunological suppression by chemical agents. Current kidney grafting techniques in man rely heavily upon chemical suppression of the immune response of the host by drugs such as Imuran and steroids. The problem is more complex in marrow grafting because the immunosuppressive drugs are toxic to the bone marrow. We have shown that methotrexate is useful in reducing the likelihood of marrow graft rejection and in ameliorating the graft-versus-host reaction. Studies are continuing to define the best dose and schedule of methotrexate. Other immunosuppressive agents are also being studied. Currently we are most interested in the anti-tumor drug, Cytoxan, which is also a potent immunosuppressive agent. We have been able to show that isogeneic marrow grafts will protect against a lethal dose of Cytoxan, and that we can secure canine allogeneic marrow grafts after Cytoxan. Studies of the effect of Cytoxan on graft-versus-host disease are planned.

7. Antilymphocyte serum. Antisera against lymphocytes induced in other species seem to offer promise as a new type of immunosuppressive agent. We have produced anti-canine lymphocyte sera in the horse. These sera have high cytotoxic antibody titers and can cause temporary lymphocyte reduction in the dog. However, these antisera vary in their ability to prolong graft survival. Their usefulness in treating graft-versus-host disease is being studied, but the results are unclear. Several laboratories have found antilymphocyte sera to be of clearcut value in graft prolongation in the rodent, but extensive additional studies will be necessary to determine its usefulness in non-rodent species including dogs and man.

Significance of Research: The studies outlined above are significant in several areas related to human disease:

BENEFICIAL APPLICATIONS OF RADIATION

GRANT NO.	TITLE OF PROJECT, RESUME & PUBLICATIONS	PRINCIPAL INVESTIGATOR & LOCATION

RH 00311
(Continued)

1. Protection against the lethal effect of irradiation. Human beings may receive large doses of irradiation by accident, through explosion of nuclear devices or during treatment of malignancies. Marrow grafting, either isogeneic or allogeneic, appears essential to prevent death at levels above 500 R whole body irradiation. Marrow grafting has evidently saved the life of one irradiation accident victim in the past year. A much broader application lies in the extension of the irradiation dose permissable for treatment of malignant diseases, particularly those such as Hodgkin's disease that may be cured by large doses of irradiation. Especially important may be the destruction of diseased marrow, such as in leukemia, and its replacement with normal marrow.

2. Transplantation of organs. We have demonstrated that long-term canine radiation chimeras will accept grafts of other organs from the marrow donor. Consistent success in marrow grafting may pave the way to successful transplantation of other organs, such as kidney and heart, without the necessity of continued administration of large doses of immunosuppressive drugs.

3. Support during marrow failure. The techniques of support for the patient with marrow failure are applicable to the clinical problem of idiopathic marrow failure, marrow failure from drug idiosyncrasy and marrow failure from large doses of anti-cancer drugs.

Publications:

Storb, R., Epstein, R. B., Buckner, C. D. and Thomas, E. D. "Treatment of Chronic Lymphocytic Leukemia by Extracorporeal Irradiation." Blood, Vol. 31, No. 4, April, 1968.

Storb, R., Epstein, R. B., Ragde, H., Bryant, J. and Thomas, E. D. "Marrow Engraftment by Allogeneic Leukocytes in Lethally Irradiated Dogs." Blood, Vol. 30, No. 6, December, 1967.

APPENDIX A

SUMMARY OF RESEARCH PROJECT GRANTS BY PROGRAM AREAS JULY 1, 1967 - JUNE 30, 1968

PROGRAM AREA	ACTIVE GRANTS	% OF TOTAL FY 1968 GRANTS BUDGET (Approx.)
METHODOLOGY OF EXPOSURE REDUCTION FOR DENTAL AND MEDICAL PRACTICES	9	11
DEVELOPMENT OF DOSIMETRY, INSTRUMENTATION AND SYSTEMS	4	3
STUDIES OF NUCLEAR PLANT MONITORING, SURVEILLANCE, AND DESIGN CHARACTERISTICS	1	1
STUDIES OF ENVIRONMENTAL PATHWAYS	3	3
STUDIES OF PROTECTIVE ACTION IN ENVIRONMENTAL CONTAMINATION	1	1
HUMAN EPIDEMIOLOGY STUDIES AND POPULATION EXPOSURE	5	12
RADIATION BIO-EFFECTS	22	19
MECHANISMS OF RADIATION DAMAGE AND EFFECTS MODIFICATION	28	30
STUDIES IN BASIC RADIATION PHYSICS AND CHEMISTRY	10	13
STUDIES OF NON-IONIZING RADIATION	4	3
BENEFICIAL APPLICATIONS OF RADIATION	2	4
TOTAL	89	100

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APPENDIX B

LISTING BY GEOGRAPHICAL AREA

STATE - COUNTRY	PAGE NO.
Alabama	210, 271
Arkansas	282
California	21, 137, 139
Colorado	101
District of Columbia	7
Florida	16, 35, 207, 217
Georgia	17, 92, 202, 253, 259
Illinois	40, 81, 154, 165, 171, 175
Indiana	43, 124, 158, 170, 211
Iowa	73, 229
Kentucky	24, 152
Louisiana	60
Maine	127
Massachusetts	15, 33, 71, 86, 120, 144, 177, 248, 263, 278, 284
Michigan	116, 126, 162, 254
Minnesota	197
Missouri	67, 129, 192, 195
New Mexico	19
New York	2, 5, 14, 16, 25, 56, 61, 79, 106, 116, 140, 144, 185, 220, 250, 260, 269
North Carolina	228, 233
Ohio	41, 238, 241, 275
Oklahoma	179
Oregon	137, 164
Pennsylvania	1, 11, 18, 45, 83, 133, 223, 237, 244, 245, 266
Rhode Island	109
South Dakota	109
Tennessee	63, 75
Texas	48, 103, 108, 132, 213, 234, 256, 281
Utah	25, 30
Virginia	97, 188
Washington	285
Wisconsin	237

STATE - COUNTRY PAGE NO.

England 87
Finland 36
Italy 93
Japan 65
Norway 148
Portugal 54

APPENDIX C

NUMERICAL LISTING BY GRANT NUMBER

GRANT NO.	PAGE NO.	GRANT NO.	PAGE NO.
RH 00005	139	RH 00253	254
RH 00008	248	RH 00268	165
RH 00021	73	RH 00272	87
RH 00030	30	RH 00279	170
RH 00039	54	RH 00280	171
RH 00042	140	RH 00293	175
RH 00061	284	RH 00294	92
RH 00065	75	RH 00297	177
RH 00076	33	RH 00298	61
RH 00077	25	RH 00302	278
RH 00081	79	RH 00303	63
RH 00089	144	RH 00304	93
RH 00095	144	RH 00305	179
RH 00098	148	RH 00307	36
RH 00099	250	RH 00311	285
RH 00124	41	RH 00312	185
RH 00140	81	RH 00316	65
RH 00175	35	RH 00318	188
RH 00193	152	RH 00321	192
RH 00204	275	RH 00322	5
RH 00206	253	RH 00325	97
RH 00210	56	RH 00328	101
RH 00211	43	RH 00330	103
RH 00218	83	RH 00345	106
RH 00222	154	RH 00347	195
RH 00239	158	RH 00350	1
RH 00243	86	RH 00354	48
RH 00245	162	RH 00358	197
RH 00246	164	RH 00364	108
RH 00252	60	RH 00366	18

GRANT NO.	PAGE NO.
RH 00367	202
RH 00379	207
RH 00384	256
RH 00386	259
RH 00390	109
RH 00391	109
RH 00392	260
RH 00394	263
RH 00395	40
RH 00400	116
RH 00403	210
RH 00404	19
RH 00405	211
RH 00412	25
RH 00416	2
RH 00422	213
RH 00428	5
RH 00429	217
RH 00430	220
RH 00431	7
RH 00433	266
RH 00434	223
RH 00437	116
RH 00443	11
RH 00450	269
RH 00455	228
RH 00461	67
RH 00464	271
RH 00467	120
RH 00470	21
RH 00473	124
RH 00474	229
RH 00479	233
RH 00482	126
RH 00483	234
RH 00487	127
RH 00494	71
RH 00496	129
RH 00499	132
RH 00501	133
RH 00506	281
RH 00507	237
RH 00512	137
RH 00520	237
RH 00521	14
RH 00523	15
RH 00528	238
RH 00529	241
RH 00530	282
RH 00532	16
RH 00536	24
RH 00537	137
RH 00545	244
RH 00548	245
RH 00562	16
RH 00563	17

APPENDIX D

ALPHABETICAL LISTING OF PRINCIPAL INVESTIGATORS

NAME	GRANT NO.	PAGE NO.
ALBERT, Roy E.	RH 00298	61
AMATO, Charles G.	RH 00428	5
ANDELMAN, Julian B.	RH 00322	45
ANDERSON, Roy S.	RH 00008	248
ARCHER, Ellen G.	RH 00496	129
BEAM, Carl A.	RH 00312	185
BLOCK, Seymour S.	RH 00429	217
BOX, Harold C.	RH 00450	269
BRODSKY, Allen	RH 00545	244
BROWNELL, Gordon L.	RH 00523	15
BUCKMAN, William G.	RH 00536	24
BURDETTE, Walter J.	RH 00483	234
CASTER, William O.	RH 00367	202
CHIPAULT, Jacques R.	RH 00358	197
CHRISTIAN, John E.	RH 00211	43
CONTE, Frank P.	RH 00246	164
COVIELLO, Dominick A.	RH 00293	175
CREGER, Clarence R.	RH 00354	48
DAVIS, Robert E.	RH 00279	170
DAVIS, Roger T.	RH 00391	109
DIMAGGIO, Anthony	RH 00252	60
FARR, Lee E.	RH 00395	40
FETNER, Robert H.	RH 00386	259
FORBES, P. Donald	RH 00390	109
FORBES, William F.	RH 00392	260
FOYE, William O.	RH 00297	177
FRANKLIN, Ralph E.	RH 00124	41
FURCHGOTT, Ernest	RH 00065	75
GARCIA, John	RH 00467	120
GHIDONI, John J.	RH 00499	132
GOEPP, Robert A.	RH 00268	165
GOLDBLITH, Samuel A.	RH 00061	284
GORIN, George	RH 00305	179
GREEN, Morris	RH 00345	106
GRIFFEN, Allen B.	RH 00487	127
HALE, John	RH 00443	11
HAYES, Robert G.	RH 00405	211
HAYMOND, Herman R.	RH 00005	139
HEMPELMANN, Louis H.	RH 00210	56
HEUSNER, William W.	RH 00400	116
HEYSSEL, Robert M.	RH 00303	63
HILARIS, Basil S.	RH 00521	14
HISATSUNE, I. C.	RH 00433	266
HORTA, Jorge da Silva	RH 00039	54
HOWARTH, John L.	RH 00404	19
JANOWER, Murray L.	RH 00494	71
JOHNSTON, Francis J.	RH 00206	253
KIMELDORF, Donald J.	RH 00537	137
KING, John S.	RH 00253	254
KORBEL, Susan F.	RH 00530	282
KRISE, George M.	RH 00330	103
KUNTZ, Robert R.	RH 00321	192
LEE, Hun	RH 00470	21
LICHTIN, Norman N.	RH 00394	263
LOHMANN, Wolfgang H.	RH 00474	229

NAME	GRANT NO.	PAGE NO.
MALKINSON, Frederick D.	RH 00280	171
MCQUADE, Henry A.	RH 00347	195
MIETTINEN, Jorma K.	RH 00307	36
MILLER, Jr., William B.	RH 00563	17
MITTLER, Sidney	RH 00140	81
MIYAGAWA, Ichiro	RH 00464	271
MIZELL, Sherwin	RH 00473	124
MONESI, Valerio	RH 00304	93
OHIRA, Masahika	RH 00316	65
OKADA, Shigefumi	RH 00437	116
OSBORNE, James W.	RH 00021	73
OWEN, Terence C.	RH 00379	207
PENDLETON, Robert C.	RH 00030	30
PIHL, Alexander	RH 00098	148
RAUSCHKOLB, Elizabeth	RH 00506	281
ROBERTS, Joan M.	RH 00482	126
ROSENTHAL, Harold L.	RH 00461	67
ROSSI, Harald H.	RH 00099	250
ROTBLAT, Joseph	RH 00272	87
ROWLANDS, John R.	RH 00384	256
RUGH, Roberts	RH 00081	79
RUPERT, Claud S.	RH 00422	213
SAVAGE, Alice M.	RH 00548	245
SAX, Karl	RH 00294	92
SAX, N. Irving	RH 00412	25
SCHUBERT, Jack	RH 00434	223
SCHUBERT, Jack	RH 00520	237
SCHULZ, Robert J.	RH 00430	220
SCHULZ, Robert J.	RH 00562	16
SHAW, Don H.	RH 00403	210
SHEER, Daniel E.	RH 00364	108
SIGLER, William F.	RH 00077	25
SLOBODIAN, Evelyn	RH 00095	144
SMITH, Edward M.	RH 00416	2
SMITH, Edward M.	RH 00532	16
SMITH, Walter T.	RH 00193	152
SOBKOWSKI, Frank J.	RH 00512	137
SPEIDEL, Carl C.	RH 00325	97
SPENCER, Herta	RH 00222	154
SPERTI, George S.	RH 00204	275
STADLER, Janice	RH 00328	101
STEINBACH, H. Burr	RH 00076	33
STRAIN, William H.	RH 00042	140
TABACHNICK, Joseph	RH 00218	83
TAYLOR, Lauriston S.	RH 00431	7
THOMAS, Edward D.	RH 00311	285
WAGNER, Richard	RH 00089	144
WANG, Richard I. H.	RH 00507	237
WANG, Yen	RH 00350	1
WARREN, Shields	RH 00243	86
WEISS, Karl	RH 00302	278
WELLER, Richard I.	RH 00366	18
WHIPPLE, G. Hoyt	RH 00245	162
WILLHOIT, Donald G.	RH 00455	228
WILLHOIT, Donald G.	RH 00479	233
WILLIAMS, Edwin G.	RH 00175	35
WILSON, Raphael	RH 00239	158
WITIAK, Donald T.	RH 00528	238
WITIAK, Donald T.	RH 00529	241
WOOD, Thomas H.	RH 00501	133
WOOLES, Wallace R.	RH 00318	188

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